THE
AMERICAN NATURALIST

VOL. LXV *May–June, 1931* No. 698

GENETIC NON-DISJUNCTIONAL FORMS IN DROSOPHILA[1]

DR. JOHN W. GOWEN

ROCKEFELLER INSTITUTE FOR MEDICAL RESEARCH, PRINCETON, N. J.

DURING the past thirteen years the writer has been interested in a stock producing forms which differ from the normal in a variety of ways, but chief among which is the enlarged pattern of the ommatidia of the eye. The abnormal flies vary within themselves. Some have a coarse eye pattern and a slightly enlarged size throughout as the chief characteristic by which they may be separated from the normal. Another group shows changes in the sex organs and secondary sexual characters, another group has the wings cut and the wing veins irregular, the fly giving a wholly debilitated appearance. A further type appears as a small fly with slender short bristles. The appearance of these types suggests the individuals which Bridges has described as triploids, sex-intergrades, supersexes and haplo IV. They differ from his material, however, in their much more frequent appearance within this stock, where all types of these individuals may appear as often as once in thirty-five times. They further differ from his material in that the abnormal forms are due in last analysis to the action of a single gene. The purpose of this paper is to describe the anatomical structure of these different forms and to compare them with the normal.

[1] From the Department of Animal Pathology of The Rockefeller Institute for Medical Research, Princeton, N. J.

THE NORMAL

Fig. 1 shows the normal male and female *Drosophila* used as parents for the production of the atypical offspring.

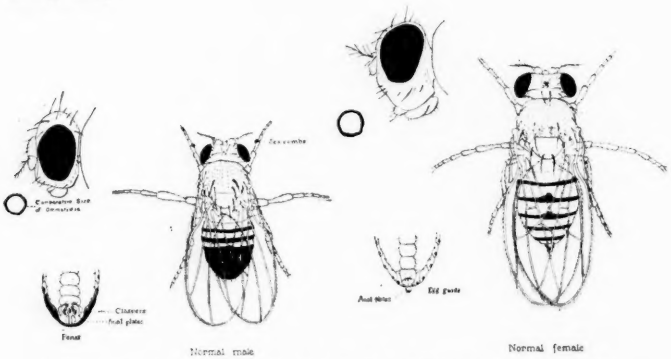

FIG. 1. The normal male and female *Drosophila melanogaster*.

The most important points of difference between the sexes visible in Fig. 1 are: the male is distinctly smaller than the female; the male has a black comb-like structure (sex-comb) on one of the joints or tarsi of the front legs, whereas these structures are lacking in the female. The abdomen of the male presents a less banded appearance than that of the female due to the difference in the distribution of pigment, although both are segmented. The external genitalia of the male are composed of plates of a shape very different from those of the female. These are known as the clasper plates and the anal plates. Those of the female are called the egg guide and anal plates. The relative size of the facets composing the compound eye is shown in the camera lucida outline of one facet.

The internal anatomy is equally distinct. The male had the following organs: two yellowish coiled tubes, the testes; two glandular structures, the paragonia, opening into the vas deferens, a duct leading from the testes and paragonia to the ejaculatory sac; the sperm vesicle,

ejaculatory sac; the ejaculatory duct leading from the sac to the penis; and the penis. The female organs are: the ovaries; ducts leading from each ovary and joining into a common duct, the oviducts; the uterus; two mushroom-shaped dark-colored organs, spermathecae, with ducts opening into the dorsal wall of the anterior end of the uterus and containing sperm after copulation; another receptacle for sperm known as the ventral receptacle; the parovaria; and vaginal portion of the uterus. The male and female flies which are the parents of the abnormal chromosome type appear to be entirely normal in respect to all these organs.

The Variant Types

Within such a group of normal individuals coming from normal parents, there occasionally appears a fly which is a variant from type in one or many particulars. The changes may affect any or all of the organs mentioned. The affected flies are generally sterile. The sex instincts are altered, the duration of life materially shortened. The first general group are individuals which have within themselves both male and female organs. These sex-intergrades may be more or less

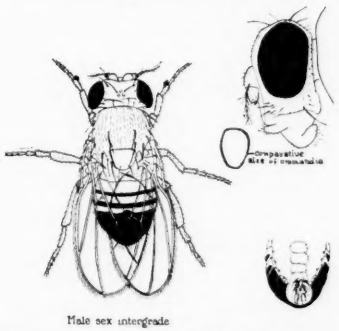

Fig. 2. Male sex-intergrade. This fly is seen to resemble closely the male externally. It differs chiefly in the pattern made by the facets of the compound eye which are large and therefore make the grid appear bigger. The outline of the facet size should be compared with the normal male as both are drawn to the same scale.

arbitrarily divided into three general types. Figs. 2, 3 and 4 show certain type-forms selected to show the extent of the changes in the external organs. These forms may be arranged arbitrarily from those most like the male to those most like the female. Fig. 2 shows the male type in appearance, the only striking change being the en-

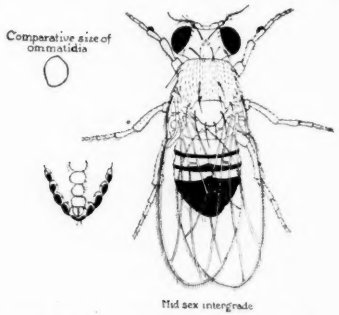

FIG. 3. Mid sex-intergrade. The noticeable features in the external appearance of individuals of this type are body appearance (sex-combs, size and pigmentation of abdominal plates) like the males while the genitalia are female. Eyes show the enlarged grid.

larged grid of the eyes formed by the increased size of the facets. The relative size of these facets is shown by the camera lucida outline of one of average size. It will be noted that the facets are much larger than those of the normal fly. Fig. 3 shows the intermediate type: the external appearance of the body being generally male, the external genitalia female, and the eyes with the enlarged grid. Fig. 4 shows the other extreme:—size of body and pigmentation of abdominal segments are of the female type. The sex-combs are generally present. Eyes show the enlarged grid. The genitalia are female type.

The types of changes which are indicated are also frequently accompanied by profound changes in behavior, both the male and female type often losing their sex instincts. These flies are uniformly sterile whether they resemble males or females in appearance. While

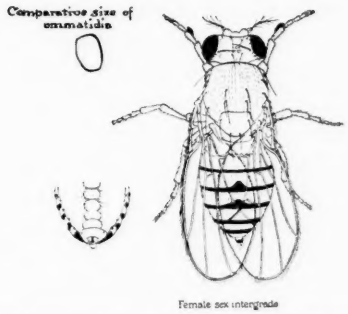

FIG. 4. Female sex-intergrade. The type of body is female with certain alterations toward the male: sex-combs and sometimes male genitalia. The eyes have the enlarged grid.

the abnormal animals have been described as type specimens, it should be understood that all intergrades between normal males and females are to be found. They are sex-intergrades in the same sense as those described by Bridges.

Besides these sex-intergrades there is another abnormal type. These flies are generally larger than the ordinary females. They are entirely normal females in appearance save the grid on the eye which is enlarged. They are fertile but are capable of reproducing markedly different progeny than those of type males and females.

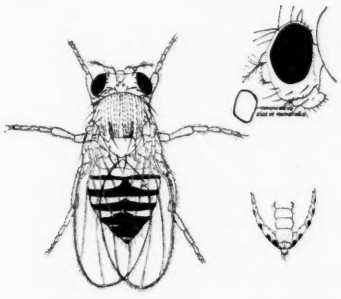

FIG. 5. Female type of fly having enlarged grid in eye pattern. Fly breeds as female reproducing normal males and females and a great excess of abnormal types shown in Figs. 2, 3, 4 and 5.

These progeny are of the following kinds; normal males and females, sex-intergrades of the three types described and females like themselves. These females are triploids.

Another type of fly with very fine eye grid and bristles is less frequently found among the progeny of these normal-appearing parents within this line of flies. These flies are physically weak and show a short duration of life. They may be of either sex. Both sexes are frequently sterile and when fertile are of low productivity. Fig. 6 shows a male of this type.

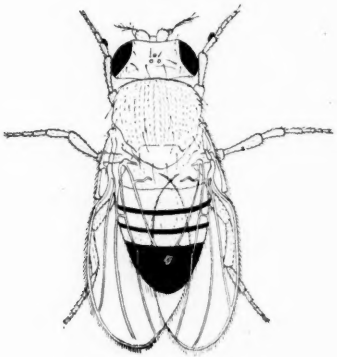

FIG. 6. Male showing slender bristles and fine eye grid.

The last type of fly found less frequently than the preceding types has a forlorn appearance, wings cut, wing veins thickened and irregular, eyes bulging with a somewhat enlarged grid, body shrunken and female throughout. Duration of life is short. These flies are sterile.

In the sex-intergrades, the internal changes are more extensive than the external changes. The male-appearing sex-intergrades tend to have organs of more nearly the male type, the mid sex-intergrades the most pronounced abnormalities, and the female sex-intergrades tending to be more nearly like the females. Thus parents 134 had a male type of sex-intergrade among their

offspring. This male sex-intergrade showed a more or less typical male appearance—sex-combs, anal plates and clasper plates. The wings were cut out, a fairly frequent change in the body of such animals. The paragonia, vas deferens, ejaculatory sac, penis and testes were present. The left testis was very small and intensely yellow. The egg guide, spermathecae, ovaries, parovaria, uterus or ventral receptacle of the female were not present.

Fig. 7 shows a drawing of the sex organs of the male sex-intergrade type. One testis is fairly normal, the other much reduced in size and of abnormal shape resembling more nearly the testis as found in the pupa. Only one paragonium of reduced size was present. Vas deferens, ejaculatory sac and penis somewhat reduced in size. Clasper plates present. Flies of this type are sterile.

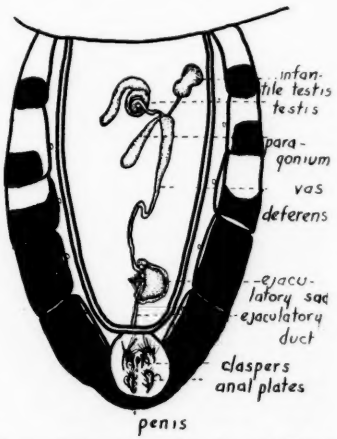

FIG. 7. Sex organs of male sex-intergrade.

In the mid sex-intergrade class, which is after all only the extreme of either class, was a fly from Female 132 which showed a general male appearance so far as the distribution of pigment on the abdominal segments was concerned. Sex-combs were present. The anal plates, egg guide, two spermathecae, ovaries (left one very

small, largely trachea), uterus and ventral receptacle of the female were present while the parovaria were absent. Of the male internal organs only a much deformed, small, yellow, right testis was present. In another such case from Female 539, the general appearance of the segments and body was female. The fly showed the following male organs: sex-combs, anal plates; one juvenile, yellow testis and one long, wrinkled, thin testis; vas deferens; and ejaculatory sac. The female organs present were egg guide, two spermathecae, parovaria, uterus and ventral receptacle. The male organs lacking were clasper plates, paragonia and penis. The female organs lacking were the ovaries.

Fig. 8 shows certain of the changes found in flies of the mid sex-intergrade type. What appeared to be a testis of the infantile type was present. No ducts or other male organs were present save the pigmentation of the abdominal plates, which was male in type. Anal plates were much deformed, clasper plates present. Two spermathecae and a ventral receptacle were present although much reduced in size. Uterus and female genitalia were present.

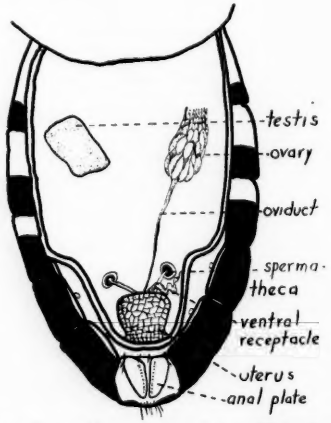

FIG. 8. Sex organs of a mid sex-intergrade. This figure should be compared with Fig. 6.

In the female sex-intergrade class there is somewhat less uniformity than in the male class. Thus one sex-intergrade from Female 102 had a general female appearance, so far as body and pigmentation of the segments were concerned. Sex-combs were present. Anal plates were much deformed, clasper plates present. Two small ovaries chiefly composed of tracheal tissue were present. Other normal organs of both sexes were absent. Another case from Female 133 had quite a normal female appearance, no sex-combs, wings cut, female anal plates, egg guide, two spermathecae, two ovaries of somewhat reduced size, parovaria, uterus and ventral receptacle. No male organs were present. In every case the eyes showed the enlarged grid.

Fig. 9 shows the generative organs as found in a female sex-intergrade. Only one ovary of much reduced size was present. The oviduct was small. Two spermathecae of small size present. The parovaria and ventral receptacle were absent. Uterus and external female genitalia present. The abdominal plates were of the female type.

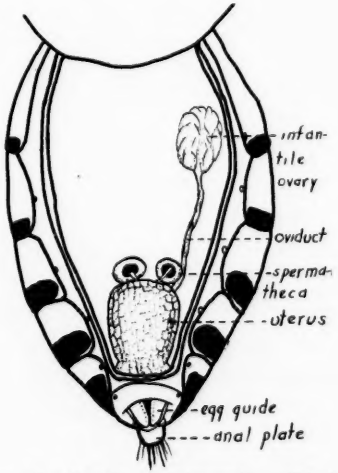

FIG. 9. Sex organs of a female sex-intergrade. This figure may be compared with Figs. 6 and 7.

TABLE I

ABNORMALITIES IN STRUCTURE OF CASE SPECIMENS

	Organ examined																
Number	Sex-combs	General appearance	Wings	Anal plates	Egg guide	Clasper plates	Spermathecae	Ovaries	Parovaria	Uterus	Ventral receptacle	Testes	Paragonia	Vas deferens	Ejaculatory sac	Penis
6278	+	♂	+	♂	–	+	–	–	–	–	–	+	–	+	+	+
6278	+	♂	+	♂	–	+	–	–	–	–	–	left + right small	+	+	+	+
6279	+	♂	+	♂	–	+	–	–	–	–	–	+ small	+	+	+	+
6296	+	♂	+	♂	–	+	–	–	–	–	–	+	+	+	+	+
6296	+	♂	+	abnormal ♂	–	abnormal +	–	–	–	–	–	+	1 small +	+	+	+
6299	1 small +	♂ +	+	♂	–	+	+	very small	–	+	–	–	–	–	–	–
6297	+	♂	+	♂	–	small twisted –	–	–	–	–	–	1 very small +	+ 1 very small	–	–	–
6296	+	♂	+	♂	–	+	–	–	–	–	–	small +	+	+	+	+
6310	+	♂	+	♂	–	+	–	–	–	–	–	+	+	+	+	+
6324	+	♂	+	♂	–	+	–	–	–	–	–	+	+	+	+	+
134	+	♂	cut	♂	–	+	–	–	–	–	–	yellow +	small +	+	+	+
117	+	♂	+	♂	–	+	–	–	–	–	–	yellow +	+	+	+	+
112	+	♂	cut	♂	–	+	–	–	–	–	–	yellow +	small +	+	+	+
114	+	♂	+	♂	–	+	–	–	–	–	–	yellow +	+	+	+	+
171	+	♂	+	♂	–	+	–	–	–	–	–	+	+	+	+	+

TABLE I—(*Continued*)

								Organ examined								
Number	Sex-combs	General appearance	Wings	Anal plates	Egg guide	Clasper plates	Spermatheeae	Ovaries	Parovaria	Uterus	Ventral receptacle	Testes	Paragonia	Vas deferens	Ejaculatory sac	Penis
115	+	♂	+	♂	–	+	–	–	–	–	–	small yellow +	+	+	+	+
133	+	♂	+	♂	–	+	–	–	–	–	–	yellow +	+	+	+	+
133	+	Mid	+	–	–	–	–	–	–	–	–	small yellow +	+	+	+	+
133	+	♂	+	♂	–	+	–	–	–	–	–	yellow +	+	+	+	+
133	+	Mid	+	–	+	–	–	small left ovary	–	–	–	small yellow right testis	–	–	+	+
132	+	Mid	+	♀	+	–	+	small left +	–	+	+	small yellow right testis	–	–	–	–
132	+	♂	+	♂	–	+	–	–	–	–	–	+	+	+	+	+
133	–	♀	cut	♀	+	–	+	+	+	+	+	–	–	–	–	–
111	+	♂	+	♂	+	–	+	–	+	+	+	2 infantile	–	–	–	–
102	+	♀	+	♀	+	mixed –	–	+ 2 small trachea tissue	–	–	–	–	–	–	–	–
102	+	Mid	+	♂	+	–	+	1 small	–	–	–	1 small yellow	–	–	+	–
205	+	♂	cut	♂	+	–	+	–	–	+	+	2 small yellow +	–	–	–	–
539	+	♀	+	♂	+	–	+	–	+	+	+	2 small yellow +	–	+	+	–
924	+	♂	+	♂	–	+	–	–	–	–	–	+	1 small +	+	+	+

A table, showing in somewhat diagrammatic form the changes found in a number of cases, is given. Histological examination of many of this type of flies frequently shows testicular and ovarian tissue present in the same organ.

The abnormal type shown in Fig. 5 has the female organs throughout. The only apparent difference between these organs and those of the normal female is that they give the impression of being slightly larger.

Flies of the type of Fig. 6 are either male or female throughout, no blending of the characters taking place. The sex organs are in general reduced in size, the flies frequently being infertile.

The last type of fly is female throughout and sterile. The ovaries and oviducts seem shrunken in appearance. Sections of the ovaries are atypical.

In their broader aspects the specimens described present a rather definite pathological picture, involving changes in the cell size, reproductive organs, etc. The group brought together as sex-intergrades are quite uniformly sterile. The type shown in Fig. 5 is fertile but shows its abnormal constitution in the fact that while breeding as a female progeny are produced which are of the normal and abnormal types.

A measure of the effect of these alterations from the normal may be had by studying the sum total effect as indicated by the time the individual's life mechanism is able to continue functioning. Experiments to determine the duration of life of each group have been performed and while of much interest in themselves need only be summarized here. The normal females live slightly longer than any other class. Individuals like those of Fig. 5 live a short time less than the normal females. The difference is not significant. The normal males live a somewhat less time. Individuals of the type of Fig. 2 have but half the duration of life of the previously mentioned classes and flies of the type of Fig. 4 live the shortest time of all, only one third the duration of life

of the other classes. These differences are all markedly significant and are of such extreme proportions as 3 to 1.

The Chromosome Structure

In the type male and female of *Drosophila melanogaster* it will be recalled that there are four pairs of chromosomes. The male has an X and Y, two pairs of long V-shaped chromosomes, the II and III, and a pair of small round chromosomes, the IV pair when seen in the metaphase plate. The female has a pair of rod-shaped X-chromosomes, two pairs of V-shaped chromosomes and a small round pair.

The normal-appearing males and females of the stock producing the sex-intergrades, triploids, slender bristled flies and super-females have been examined for their chromosome structure. Drawings of typical plates are shown in Fig. 10.

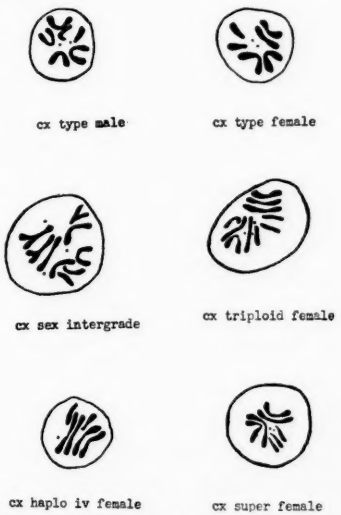

Fig. 10. The chromosomes of the male and female *Drosophila*, cx. stock, and sex-intergrades, triploid females, haplo IVs and super-females appearing as progeny from them.

The chromosome figures of the normal-appearing flies within this particular stock are seen in Fig. 10 to be essentially the same as those of the ordinary wild type stocks.

The female chromosomes differ from those of the male in that the female normally has two X-chromosomes and no Y-chromosome. Otherwise the female chromosomes II, III and IV are the same as those of the male. This is the most typical case of this particular stock. Occasionally the female cells show besides the two X-chromosomes a Y-chromosome like that of the male. This Y-chromosome appears to play no particular part in the further life of the fly; in fact, is simply a concurrent mechanical condition brought about by the atypical maturation processes of the egg formation. The normal males and females of this stock are therefore essentially like the males and females of wild types.

The cells of the sex-intergrades, figures 2, 3 and 4, show a triploid instead of a diploid complex, there being twelve chromosomes present instead of eight, two X-chromosomes, a Y-chromosome and three each of the second, third and fourth chromosomes. The fourth chromosomes are not always clear in all the figures, as they are small and difficult to differentiate. Fig. 10 shows the chromosome plate of such a form.

The changes which are found in flies of the type of Fig. 5 are similar to those in the other forms. Fig. 10 shows the chromosome plate of such a fly. The chromosomes are in triplicate throughout: three X's, three II's, three III's and three IV's, with occasionally a Y-chromosome. This arrangement of the chromosomes enables this fly to form normal eggs and diploid eggs. The dual nature of the eggs from these flies results in a repetition in the progeny of the types previously described, normal males and females, sex-intergrades and triploid females. This repetition of the different types in the progeny of such triploid females is proof that the abnormal chro-

mosome number is the underlying cause which is responsible for the abnormalities found.

The slender bristled flies are found to have a normal chromosome constitution, save in one particular; they have only one fourth chromosome. The low viability of such flies makes this difficult of proof in every case, but both the cytological figures found and the genetic tests possible all substantiate this conclusion. They apparently correspond to Bridges' haplo IV.

The debilitated females are triploid in the sex chromosomes but diploid otherwise. They are apparently identical to super-females.

The Gene as the Etiological Agent

Since the purpose of this paper is simply to present the anatomical aspects of the problem, it need only be said that the physical entity causing these extensive changes, when viewed grossly or microscopically, is a gene in the middle of the third chromosome.

Discussion

The sex-intergrades, triploids, slender bristled weak males and super-females, as herein described, come directly from normal parents. A single pair of these normal flies may give 1 to 6 abnormal flies during the course of the culture, seldom more than that. These atypical flies give the first inkling of the abnormal conditions in the maturation processes of the eggs within the parents of this particular strain. All these atypical forms have an abnormal chromosome number within each of their cells. The extensive alterations from the well-known wild-type fly are due to the fact that these mothers gave them eggs having their peculiar chromosome numbers. The flies may be considered as primary non-disjunctional forms in which we are observing the origin of new types. The types described by Bridges, since they come from and as a mechanical consequence of this primary non-disjunction, are secondary non-dis-

junctional forms. It is of interest therefore to note the similarity of these individuals with the secondary non-disjunctional triploids, sex-intergrades, super-females and minute bristled flies as described by Bridges and by Dobzhansky and Bridges. So far as can be determined the types are identical, type for type, in their external appearance, internal structure and chromosome characteristics and even to the variability observed in the organ distribution of the intersexes.

It is, however, possible to obtain all types of non-disjunctional forms as primary individuals from this stock, whereas the secondary forms are less likely to be found all within one strain.

Within this strain the frequency of the formation of these primary non-disjunctional individuals varies from as low as 1 to 100 flies to as high as 1 to 30. This ratio is 100 to 300 times the rate found in ordinary stocks. Bridges has made the suggestion that when normal stocks have such an occasional primary non-disjunctional individual found in the progeny that such an individual comes from cells within the ovary which are markedly larger than the ordinary cells and carry more than the ordinary complement of chromosomes. This reasoning is based on the evidence that "in three separate preparations of ordinary 2 N females it was found that a portion of an ovary was constituted of markedly larger cells; and in two of the individuals some of the giant cells were in division, and the chromosomes could be counted as 4 N. Evidently there had been in some oogonial cell a division of the chromosomes that had not been followed by division of the nucleus and cytoplasm. The resulting tissue was tetraploid, and any reduced gamete would be 2 N. Such a 2 N gamete, fertilized by a normal sperm, would account for each of the twenty-five recurrences of triploid." In view of the increased frequency of the abnormal forms within the strain here described it would be expected that, if the hypothesis of Bridges were true, areas of large-sized cells and multiple chromosome num-

bers would be quite common within the ovaries of females from this strain. Such is found not to be the case. No enlarged cells have been observed within the ovaries of the normal parents. Neither have cells been observed with increased chromosome numbers. The evidence of the number of abnormal types per parental bottle furthermore point rather to the maturation division as the point where the non-disjunction takes place in forming these atypical chromosome flies at least for this stock.

The data herein presented support the contention that sex is determined as an interaction of the autosomal chromosomes and the sex chromosomes. The fact that within the intersex group there is such a wide variation in the degree with which the sex characters are developed and that this variation seems to be correlated with the type of gene complex found in the chromosomes further points to the conclusion that the gene elements within the chromosomes are the responsible factors and not the chromosome *per se*.

The cause of these changes is a third chromosome factor. To the extent that this factor modifies the end product of the reaction sex it could be looked upon as a sex factor. It is, however, probably better since it is one of the very few genes for which we have some information of its physical action to regard it rather as it really is—a factor whose presence or absence materially influences the maturation of the chromosomes within the female.

The cell size of the ommatidia of the eyes is shown beside the figure of the fly. Study of these brings out the fact that the cell size is correlated directly with the chromosome quantity contained within them. Dobzhansky's extensive study of this same question utilizing the cell size of the wing arrives at the same conclusion for the secondary non-disjunctional forms.

The recent republication of Boveri's hypothesis of the cause of cancer as due to abnormal chromosome numbers within the cancer cells and Metcalf's revival of this

hypothesis in his writings makes a consideration of these results from that view-point significant, since within the cells of the forms here described there is chromosome reduplication and chromosome unbalance. In outline Boveri's suggestion is based on the following reasoning: By fertilization of sea-urchin eggs with sperm in various ways abnormal chromatin forms could be produced. The cell size of these forms bore a correlation to the amount of chromatin contained in their nucleus. Mitoses were frequently abnormal. The number of chromosome combinations was very great, rivaling the number of types of tumors. Amitoses took place. The chromosome complex, incompatible in number, kind, or both, clearly caused these abnormal structural forms. As a parallel to this, cancer cells are frequently associated with enlarged cell size brought about by nuclear fusion. These cells may form irregular spindles and eventually pass into amitotic division. On this parallel Boveri based his suggestion. In a more general perspective, the case appears even stronger, for as Wilson points out the limitations of growth and cell-division, while they may be controlled by a variety of agents, such as available food and hormones, are in the last analysis governed by heredity. This is obvious from the fact that specific mean size of organisms is hereditary. Histological analysis has shown that it is the number of cells rather than their size which governs an organism's mass. Mendelian analysis has shown that this size is determined by the genes within the chromosomes. Or speaking more broadly, the limits of cell-division and, therefore, growth are determined by the chromosome constitution of the cells. The mechanism controlling the amount of cell-division is therefore present in the cell itself and should it be released might well give the uncontrolled cell multiplication so impressive in tumor formation.

The facts deduced from this investigation are of particular significance to this hypothesis of Boveri. In this

particular stock fertilized eggs are produced which in turn give rise to individuals which have 3 sex-chromosomes and 3 of each autosome group. While these individuals show a slightly larger cell size and body mass than the normal females of 2 sex-chromosomes and 2 of each autosome group, they are otherwise typically female in their organs, no noticeable abnormalities appearing. From this, the conclusion appears justified that the mere quantitative multiplication of the chromatin does not necessarily cause abnormal growths. The other type of abnormal eggs contains 3 sex-chromosomes, two of the X-type and one Y-chromosome, and 3 of each autosome. These individuals differ from both the normal male complex (one sex-chromosome of the X-type and one of the Y-type and 2 of each of the autosomes) and the normal female complex (2 sex-chromosomes of the X-type and 2 of each autosome group). Such adults are thus different in both the quantity and balance of the chromatin from the normal individuals. These individuals present abnormalities which are of wide range and clearly marked but they seem to result in a restraint in growth rather than a complete lack of its control. In fact, the abnormal organs formed and their peculiar association, *i.e.*, ovary and testis in the same animal or spermathecae associated with testes, are in general much reduced in size. In the germ cells, cell division and the normal maturation process appear hampered, but few cells going through the complete processes to egg or sperm formation, sterility due to this excessive cell restraint apparently always resulting. These results, therefore, tend to negative Boveri's suggestion if they are accepted as comparable material on which a test of his hypothesis could be made. These organisms do have a characteristic which might conceivably produce a difference in reaction from that Boveri postulates, namely, they presumably have all their cells with the given abnormal chromosome complex. The result might well be different if only certain of the cells were ab-

normal in their chromosomes and growing perhaps at the expense of the rest. Such cases, known in *Drosophila* as gynandromorph and mosaic flies, have areas which differ from the rest of the body areas in the number of their chromosomes without the production of abnormal growth. The results agree with Boveri's in showing that the chromosome complex markedly influences growth.

SUMMARY

This paper presents an analysis of the type parents and atypical forms found within a special stock of *Drosophila melanogaster*. The anatomical changes observed in the abnormal individuals of this stock consist very largely in over or under growth of the organs, especially those of the reproductive system, bristles and wings. They involve alterations in the testes, ovaries, paragonia, vas deferens, ejaculatory sac, oviduct, spermathecae, parovaria, ventral receptacle and uterus, as well as the external genitalia, sex-combs, clasper plates and anal plates. Fertility is reduced. The duration of life is shortened. Bristles are reduced and wings cut. These forms appear sporadically within a certain family or its near relatives. The ratio of the abnormal individuals to normal varies anywhere from 1 in 26 to 1 in 100. Profound alterations are found within the cells of these abnormal individuals. The chromosomes are increased in number, 12 chromosomes instead of 8 being ordinarily found in the cells. The anatomical changes within the abnormal flies are shown to be associated with the alterations of this chromosome number. The agent which brings about these changes is a third chromosome recessive gene.

LITERATURE CITED

Boveri, Th.

1905. Zellen-Studien, Heft 5. "Ueber die Abhängigkeit der Kerngrösse und Zellenzahl der Seeigel-Larven von Chromosomenzahl der Ausgangszellen," Jena, 1905, Gustav Fischer.

Boveri, Th.
1907. *Idem.* Heft 6. "Die Entwicklung dispermer Seeigel-Eier. Ein Beitrag zur Befruchtungslehre und zur Theorie des Kerns," Jena, 1907, Gustav Fischer.

Boveri, Th.
1914. "Zur Frage der Entstehung maligner Tumoren," Jena, Gustav Fischer.

Bridges, C. B.
1921. "Proof of Non-Disjunction for the Fourth Chromosome of *Drosophila melanogaster*," *Science*, n. s., liii: 308.

Bridges, C. B.
1921. "Genetical and Cytological Proof of Non-Disjunction of the Fourth Chromosome of *Drosophila melanogaster.*" Proceedings of the National Academy of Sciences, VII: 186–192.

Bridges, C. B.
1921. "Triploid Intersexes in *Drosophila melanogaster*," *Science*, n. s., liv: 252–254.

Bridges, C. B.
1922. "The Origin of Variations in Sexual and Sex-limited Characters," AMERICAN NATURALIST, lvi: 51–63.

Bridges, C. B.
1925. "Sex in Relation to Chromosomes and Genes," AMERICAN NATURALIST, lix: 127–137.

Dobzhansky, Th.
1929. "The Influence of the Quantity and Quality of Chromosomal Material on the Size of the Cells in *Drosophila melanogaster*," Wilhelm Roux' Archiv für Entwicklungsmechanik der Organismen, cxv: 363–379.

Dobzhansky, Th., and Bridges, C. B.
1928. "The Reproductive System of Triploid Intersexes in *Drosophila melanogaster*," AMERICAN NATURALIST, lxii: 425–434.

Gowen, John W.
1928. "Mutation, Chromosome, Non-Disjunction and the Gene," *Science*, lxviii: 211–212.

Gowen, Marie S., and John W.
1922. "Complete Linkage in *Drosophila melanogaster*," AMERICAN NATURALIST, lvi: 286–288.

Metcalf, N. M.
1926. "Boveri on Cancer," *Science*, n. s., lxiv: 499–500.
1928. "Cancer (?) in Certain Protozoa," *American J. Trop. Med.*, viii: 545–557.

THE DECREASE OF CROSSING-OVER OBSERVED IN TRANSLOCATIONS, AND ITS PROBABLE EXPLANATION

TH. DOBZHANSKY

CALIFORNIA INSTITUTE OF TECHNOLOGY

A TRANSLOCATION is a rearrangement of chromosomal material involving the transfer of a section of a chromosome from its normal location to a new locus. The first translocation observed in *Drosophila melanogaster* (Bridges, 1923), as well as most of the translocations described in this animal since then, are simple translocations. In simple translocations a chromosome is broken, and one of the resulting fragments is attached to a different chromosome, the recipient chromosome being intact. Recently also reciprocal translocations have been found in *Drosophila* (Sturtevant and Dobzhansky, 1930; Muller, 1930; unpublished data of Karpechenko and of Dobzhansky). In these cases two chromosomes were broken, and the resulting four fragments reunited so as to give two "new" chromosomes. The phenomenon of reciprocal translocation is evidently identical with that known as segmental interchange. Segmental interchange is described by Belling and Blakeslee in *Datura* (Belling, 1927; Blakeslee, 1927*a*, 1927*b*, 1929), by Burnham (1930) in maize, and postulated to occur in Oenothera and in certain other plants (Darlington, 1929; Cleland and Blakeslee, 1930; Blakeslee and Cleland, 1930; Håkansson, 1920; Meurman, 1929).

The transposed fragment of the donor chromosome is in most cases attached to the end of the recipient chromosome (Patterson and Muller, 1930; Dobzhansky, 1930*a*). Cases, however, are known in which a fragment of the donor chromosome becomes attached to the side of the recipient chromosome at a point between the spindle

fiber and the end of the recipient (Bridges, 1923; Hamlett, 1926; unpublished data of Sturtevant). In these cases a branched chromosome is formed (such a branched chromosome, however, has not been observed cytologically).

Of the two fragments formed by breakage of a chromosome, it is the one which does not include the locus of the spindle fiber attachment which becomes reattached to a different chromosome. The other fragment of the donor chromosome, which includes the locus of the spindle fiber, may remain free, or a fragment of some other chromosome may become attached to it. Thus, the formation of chromosomes having no spindle fiber attachment or more than one does not occur, or, more probably, such an event leads to formation of a chromosome which is unable to behave normally in mitosis, and is therefore eliminated.

In most of the translocations known, the fragment of the donor chromosome transposed onto the recipient chromosome includes one of the free ends of the donor. Recently at least two cases have been found in which sections taken from the middle of the second chromosome became attached to the Y-chromosome (Dobzhansky, 1930*b*, unpublished data of Rhoades and of Schultz), and the parts of the second chromosome lying to the right and to the left of the removed section reunited again. This kind of translocation is possibly related to the phenomenon of deletion (Painter and Muller, 1929; unpublished data of L. V. Morgan and of Dobzhansky). In the known deletions, sections of the X-chromosome not involving either end of the chromosome were lost, and the two ends reunited again, forming a new chromosome which is both genetically and cytologically shorter than the normal X-chromosome of *Drosophila melanogaster*.

Individuals carrying translocations in heterozygous form are in most cases phenotypically normal. This fact stands in accord with expectations, since flies carrying translocations have all the genes present in normal

flies. Some of the translocations, however, are different from normal flies in appearance. The "Pale" translocation of Bridges (*l.c.*) produces a dilution of the eosin eye-color. A reciprocal translocation recently found by the present author (unpublished) affects the eye-shape in a manner similar to the well-known gene Bar. This translocation represents an exchange of section between the second and X-chromosomes. The factor producing this change of eye-shape, called baroid, behaves as a recessive allelomorph of Bar, and its locus apparently coincides with the point at which the X-chromosome has been broken. Such a coincidence of the locus of mutation with the locus of the breakage of a chromosome is noteworthy. The fact that the majority of translocations, at least in *Drosophila,* are lethal when homozygous (Muller and Altenburg, 1930; Dobzhansky, 1930*a*), and that the lethal factors seem to be located usually at or very near the breakage point, suggests some connection between certain mutations and chromosomal breakages. However, some translocations in *Drosophila* are fully viable and normal in appearance in homozygous condition (Dobzhansky, 1929; also unpublished data).

It is obvious from the account given above that the phenomena classed as translocations are very diverse in nature. The study of translocations in *Drosophila,* however, shows that one property is common to most of them. This is the reduction of the frequency of crossing-over in the chromosome, or in the limb of a V-shaped chromosome, in which the breakage occurred, or in the chromosome or in the limb of chromosome to which a fragment of another chromosome becomes attached. The following review of the known translocations in *Drosophila melanogaster* shows how widely this effect on crossing-over is distributed.

The reduction of crossing-over is observed in flies that are heterozygous for translocations. Individuals heterozygous for a translocation carry a chromosome broken into two parts (the donor chromosome, A^2 and A^3 in Fig.

1), and a normal (*i.e.*, unbroken) homologue of this chromosome (A^1, Fig. 1). One of the two parts of the

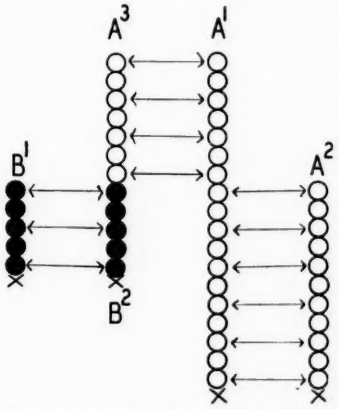

FIG. 1. A scheme of attraction between chromosomes involved in a translocation. The donor chromosome and its homologue are represented in white, the recipient chromosome and its homologue are in black. The locus of the spindle fiber is marked by ×. A^1—the normal homologue of the donor chromosome. A^2—the part of the donor chromosome preserving its own spindle fiber attachment. A^3—the part of the donor chromosome attached to the recipient chromosome. B^1—the normal homologue of the recipient chromosome. B^2—the recipient chromosome. The arrows indicate the direction of the attraction forces between the homologous loci in different chromosomes.

donor chromosome, which does not include the locus of the spindle fiber attachment, becomes attached to the recipient chromosome (B^2, Fig. 1). The other part of the donor chromosome (A^2), which includes the locus of the spindle fiber attachment, remains free. Crossing-over may occur between either section of the donor chromosome and its normal homologue. That is to say, crossing-over may occur between A^3 and the corresponding part of A^1, as well as between A^2 and the corresponding part of A^1. Sometimes double crossovers are observed in which the crossing-over occurred in both sections of the donor chromosome simultaneously. The appearance of such doubles proves that both parts of the

donor chromosome may conjugate with the normal chromosome simultaneously.

Five translocations involving the third and fourth chromosomes were studied in detail (Dobzhansky, 1930*a*). In all of them a section of the third chromosome was broken off and attached to the fourth chromosome. In three cases (*a*, *b*, *e*, see Table I) the third chromosome was broken between the spindle fiber (which is located at the locus of the gene peach, in the st – cu interval) and the left end (the ru end) of the chromosome. In these three cases a pronounced reduction of crossing-over was observed in the left limb of the chromosome (*i.e.*, between ru and peach), while in the right limb of the chromosome crossing-over remained normal, or was even slightly increased (see Table I). In two other cases (*c* and *d*, Table I) the third chromosome was broken to the right of the spindle fiber, and a reduction of crossing-over is observed in the right limb of the chromosome.

Four translocations involving a transfer of a section of the second chromosome to the fourth were found

TABLE I

DIFFERENCES BETWEEN THE CROSSING-OVER FREQUENCIES OBSERVED IN THE THIRD CHROMOSOME IN TRANSLOCATIONS, AND THE FREQUENCIES OBSERVED IN CONTROL

Interval		Control value	Translocations				
			a	*b*	*c*	*d*	*e*
Left limb	ru–h	23.7	– 6.5	– 2.5	+ 3.2	+ 5.4	– 4.2
	h–D	13.7	–10.8	– 7.8	+ 1.7	+ 1.1	– 2.1
	D–th	1.1	– 0.9	– 0.9	+ 0.4	0.0	0.0
	th–st	0.8	– 0.6	– 0.5	+ 0.1	+ 0.1	– 0.3
Spindle fiber	st–cu	8.0	– 1.4	– 3.4	– 3.5	– 2.1	– 2.9
Right limb	cu–sr	15.4	+ 1.2	+ 1.7	–11.7	– 2.8	+ 0.2
	sr–e^s	10.3	+ 1.4	– 0.3	– 2.8	– 1.4	+ 1.3
	e^s–ca	31.0	0.0	0.0	– 2.1	–11.8	0.0

(Dobzhansky, 1930*b*). In two of them (*a* and *d*, Table II) the breakage occurred in the right limb of the second chromosome, and a striking reduction of crossing-over is observed in this limb, while the crossing-over in the left limb is about normal. In two others (*b* and *c*, Table II) the breakage took place to the left of the spindle fiber (the spindle fiber in the second chromosome is attached at the locus of the gene pr), and a strong reduction of the frequency of crossing-over is observed in the left limb, while the right limb of the chromosome shows normal crossing-over. It is worth noticing that in all these cases the relatively strongest reduction of crossing-over is observed near the point of breakage of the chromosome (in the pr – c interval in *a*-translocation, in the c – px interval in *d*-translocation, see Table II), but in all cases some decrease of the frequency of crossing-over is observed in the whole limb in which the locus of breakage lies, and never in the opposite limb.

Four translocations involving the second and third chromosomes were studied (Sturtevant and Dobzhansky, 1930; Dobzhansky and Sturtevant, in press). In two of these translocations both the second and third chromosomes were broken at the spindle fiber, the left limb of the second and the left limb of the third chromosome united to form one "new" chromosome, and the two right limbs formed another "new" chromosome. Crossing-over was found to be decreased throughout the second and third chromosomes. However, if crossing-over in the second chromosome of these translocations is prevented by the crossover suppressors $C_{II\,L}$ and $C_{II\,R}$, the third chromosome shows the normal amount of crossing-over. Likewise, if crossing-over in the third chromosome in these translocations is suppressed by $C_{III\,L}$ and $C_{III\,R}$, crossing-over in the second chromosome remains normal.

In the third of these translocations the third chromosome is broken at the spindle fiber, but the second chromosome is broken to the left of its spindle fiber.

The right limb of the third chromosome is attached to the longer fragment of the second, and the shorter fragment of the second chromosome is attached to the left limb of the third. A strong reduction of crossing-over is observed in the left limb of the second chromosome, some reduction in the whole third chromosome, but the right limb of the second chromosome shows normal crossing-over. In a fourth translocation a section of the left limb of the second chromosome is attached to but not at the end of the left limb of the third. A striking reduction of

TABLE II

DIFFERENCES BETWEEN THE CROSSING-OVER FREQUENCIES OBSERVED IN THE SECOND CHROMOSOME IN TRANSLOCATIONS, AND THE FREQUENCIES OBSERVED IN THE CONTROL

Interval		Control value	Translocations			
			a	*b*	*c*	*d*
Left limb	al–dp	13.6	− 3.0	−13.3	−13.3	+ 0.1
	dp–b	31.0	− 0.2	−30.2	−21.4	+ 1.5
	b–pr	8.5	+ 0.1	− 8.0	− 2.4	+ 0.7
Spindle fiber						
Right limb	pr–c	21.3	−19.5	− 1.9	+ 5.1	− 7.6
	c–px	23.8	−19.0	+ 2.8	+ 6.5	−13.8
	px–sp	7.1	− 3.3	+ 1.3	− 0.2	− 4.5

crossing-over is observed in the left limb of the second and in the left limb of the third chromosome, but crossing-over is normal in the right limbs of both chromosomes.

In the "Pale" translocation of Bridges (1923) a relatively small section of the right limb of the second chromosome is broken off, and attached to the right limb of the third chromosome, again not at the end of the chromosome. Hamlett (1926) found that crossing-over is reduced in the right limb of the third chromosome, but normal in the left limb of the same chromosome. No

data on the crossing-over in the second chromosome in this translocation are so far available.

Anderson (1929) has described a strain in which a high frequency of non-disjunction of the X-chromosomes is observed. According to unpublished data of Anderson, the high non-disjunction in this strain is due to the presence of a translocation involving the X- and third chromosomes. The locus of this translocation in the third chromosome is in the Dalta-Hairless region, *i.e.*, in the right limb. Anderson found a reduction of crossing-over in the X-chromosome, and in the right limb of the third chromosome.

The present author has found a translocation involving the X- and second chromosomes. The X-chromosome is broken at the locus of Bar, and the second chromosome is broken at vestigial (*i.e.*, in the right limb), and the broken-off fragments of both chromosomes are exchanged. A reduction of crossing-over is observed in the right limb of the second chromosome, and at least in a part of the X-chromosome. The left limb of the second chromosome shows normal crossing-over.

Ten translocations involving the second and Y-chromosomes were found (Dobzhansky, 1930*b*, also unpublished data). In five of them a relatively small section of the right or the left limb of the second chromosome (including one of the ends of the chromosome) is broken off, and attached to the Y-chromosome. A reduction of crossing-over is found in every case in the limb of the chromosome in which the breakage took place, but nearly normal amount of crossing-over is observed in the opposite limb. In two translocations a section including the second-chromosome gene purple and some of the neighboring loci, but not including either end of the second chromosome, was transposed onto the Y-chromosome. In one of these translocations (studied by Mr. M. Rhoades) a reduction of crossing-over is found in the left limb of the second chromosome (in which the gene purple is located), but not in the right limb of the same

chromosome. In another case (studied by Dr. J. Schultz) some reduction of crossing-over is observed in the left limb of the second chromosome, but in the right limb crossing-over is nearly entirely suppressed. This nearly complete suppression is, probably, due to the presence of an inverted section, which arose simultaneously with the translocation. Finally, in three of the translocations involving the second and Y-chromosomes, nearly the whole limb of the second chromosome is broken off and reattached to the Y. In these three cases crossing-over in the second chromosome is normal.

According to Muller (1928*a*, 1928*b*) reduction of crossing-over is observed at least in some of the numerous cases of translocations studied by him. Oliver (1930) used the reduction of crossing-over as a method of distinguishing the gene mutations from chromosomal aberrations, including translocations.

The facts presented above may be summarized as follows: (1) If a chromosome is broken between its spindle fiber and its end, or if a fragment of another chromosome is attached to it, a reduction of the frequency of crossing-over in this chromosome is observed. (2) The relatively strongest reduction is observed in the intervals in or at which the breakage or the attachment took place. (3) In V-shaped chromosomes crossing-over in one limb need not be influenced by events taking place in the opposite limb. (4) If a V-shaped chromosome breaks into two equal parts (*i.e.*, if the breakage occurs near the spindle fiber), crossing-over in the resulting fragments may or may not be affected.

Several mechanisms may be suggested to explain these relations. It may be supposed, for instance, that an inverted section arises concomitantly with every translocation, and produces the observed change in the frequency of crossing-over. Such a situation may take place in some cases, but there is no known reason why inversions must always, or nearly always, arise together with translocations. Furthermore, reduction of cross-

ing-over is observed not only in case of breakage of chromosomes, but also when a section of a chromosome becomes attached to another, apparently intact, chromosome. Hence, an assumption would have to be made that a chromosome may become a recipient of a fragment of another chromosome only if it happens to carry an inversion. Finally, it is very doubtful whether an inverted section lying in a broken-off fragment of a chromosome can produce a decrease of the crossing-over frequency in the remaining part of the donor chromosome. Likewise, an inverted section lying between the spindle fiber and the locus of breakage can hardly be supposed to affect crossing-over in the broken-off fragment. The facts show, however, that crossing-over is reduced in the whole limb in which breakage took place. Thus, the inverted-sections hypothesis must be discarded.

The breakage of the chromosome, producing a change in the normal alignment of the chromosomal material, may be suggested to be the cause of the disturbance of crossing-over. This explanation is put aside by the results of the experiments of Rhoades (in press). Rhoades studied crossing-over in females having two normal second chromosomes, and a fragment (duplication) of second chromosome attached to the Y-chromosome. The presence of this fragment causes a reduction of crossing-over in the normal second chromosomes, which are unaffected by breakage. A similar result was obtained by the present author. Crossing-over was studied in females having two normal X-chromosomes, and, in addition to that, a fragment of X-chromosome (a deleted X-chromosome). Crossing-over in the normal X-chromosomes was found to be decreased as compared with a control experiment.

One may suppose that normal pairing of chromosomes, leading to normal crossing-over, may take place only if chromosomes first pair at the spindle fiber attachment. If so, the reduction of crossing-over in the broken-off section of a chromosome, which has a "new" spindle

fiber attachment provided by the recipient chromosome, becomes understandable. But this hypothesis does not explain the reduction of crossing-over which is observed between the locus of breakage and the spindle fiber in the donor chromosome. If the supposition is made that the ends of chromosomes determine the normal synapsis and crossing-over, a similar difficulty is encountered. For in this case reduction of crossing-over may be expected to occur in the donor chromosome, but not in the broken-off fragment. Moreover, neither of these hypotheses explains the reduction of crossing-over in the recipient chromosome, provided the latter is not broken.

A hypothesis, however, may be suggested which seems to fit the available data on reduction of crossing-over in translocations. An assumption must be made that the normal pairing of chromosomes, which is a prerequisite of normal crossing-over, is caused by the homologous loci (genes) in the chromosomes exhibiting at a certain moment of gametogenesis a specific attraction to each other.

Fig. 1 represents the donor chromosome A^2, a section of which A^3 is broken off and attached to the recipient chromosome B^2. An unbroken homologue of the donor chromosome, marked A^1, and a homologue of the recipient chromosome, marked B^1, are present in the same cell. The donor chromosome A^2 and its homologue A^1 are represented in Fig. 1 in white, the recipient chromosome and its homologue B^1 and B^2 are in black. The arrows show the attraction exhibited by the homologous loci.

Although the mutual attraction is ascribed to genes and not to chromosomes as wholes, chromosomes are known to be coherent bodies, and therefore must exhibit a certain degree of tenacity. Hence, the forces which pull a part of a chromosome in a given direction may be interfered with by other forces which may pull another part of the same chromosome in a different direction, and this is the situation in which certain chromosomes are in translocations. Chromosome A^2 is attracted

toward A^1. The chromosome A^1, however, is attracted to A^2 as well as to A^3. If A^1 happens to lie between A^2 and A^3, a conflict of the attraction forces is at hand. Hence, pairing of A^1 with either A^2 or with A^3, or with both, may be retarded, or may never take place. The chromosome B^2A^3 will be in a similar situation if it happens to lie between A^1 and B^1 (see Fig. 1). Indeed, in this case the chromosome A^3B^2 is being pulled in two opposite directions at the same time. This scheme (Fig. 1), therefore, gives a graphic representation of a situation which may lead to reduction of the frequency of crossing-over between the spindle fiber and the locus of breakage in the donor chromosome (the locus of the spindle fiber is represented in Fig. 1 by a cross mark), as well as in the broken-off section (A^3), and in the recipient chromosome (B).

An objection might be made that the relative position of chromosomes represented in Fig. 1 is a rather special case, and that in other positions of chromosomes no conflict between different attraction forces arises. This is partly true, but if one takes into account the fact that at the moment of pairing the chromosomes are distributed in the nucleus in a three-dimensional space, this objection loses most of its force. For if chromosomes are represented lying in three dimensions, there are relatively few geometrical positions in which a conflict of the attraction forces is entirely eliminated. Moreover, in none of the translocations is crossing-over reduced to nil, which fact proves that in some cases chromosomes involved in translocations do pair successfully. It must be emphasized that presence of at least some crossing-over in all intervals in chromosomes involved in translocations constitutes a distinction between translocations and inversions. Indeed (Sturtevant, 1926, also unpublished data), no single crossing-over in the inverted sections of chromosomes can be recovered.

The reduction of crossing-over produced by inversions of sections of chromosomes can also be represented as

the result of a conflict of attraction forces. Sturtevant suggested in 1921 (Sturtevant, 1921) that if a section of a chromosome is inverted, and if a chromosome carrying an inversion is present in heterozygous condition (*i.e.*, along with a homologous chromosome having a "normal" sequence of genes), a reduction of crossing-over must result. In individuals which are homozygous for the inverted section the frequency of crossing-over must be normal. Inverted sections were actually found in *Drosophila* (Sturtevant, 1926, also a paper in press), and their genetic behavior was found to be in accord with expectations.

Fig. 2 represents a chromosome (marked A) carrying

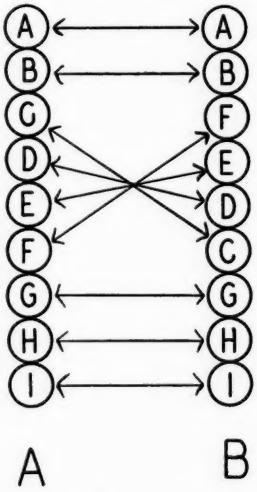

FIG. 2. A scheme of attraction between chromosomes differing from each other by an inverted section. CDEF—the inverted section. The arrows indicate the direction of the attraction forces between the homologous loci.

the genes ABCDEFGHI. If the CDEF section of this chromosome becomes inverted, a chromosome ABFEDCGHI arises (B, Fig. 2). The arrows show the direction of the attraction forces, which tend to

bring the gene A in contact with A, the gene B with B, C with C, etc. It follows from the inspection of Fig. 2 that in any geometrical position which chromosomes ABCDEFGHI and ABFEDCGHI may occupy in respect to each other some parts of one chromosome will be pulled away from the homologous parts of the other chromosome. It also follows from Fig. 2 that some reduction of crossing-over must be observed not only within the inverted section, but between the inverted section and the spindle fiber, and between the inverted section and the free end of the chromosome (*i.e.,* in the AB and in the GHI regions) as well. This is in agreement with experimental evidence secured by Sturtevant (*l.c.*).

The reduction of the frequency of crossing-over produced by breakages, by fragments of other chromosomes either free or attached, and by inverted sections is strongest in the neighborhood of the breakages, attachments and inverted sections, and becomes less pronounced in the parts of the chromosomes remote from the loci of these disturbances. This fact may be considered as a corollary of the attraction hypothesis. Indeed, the greatest conflict of the attraction forces must occur in the vicinity of the region of the chromosome which carries physical particles which are being pulled in opposite directions (see Figs. 1 and 2).

The effect of the inverted sections and translocations on crossing-over seems to be restricted to that limb of a V-shaped autosome of *Drosophila* in which the inversion or the translocation took place. The opposite limb of the same chromosome is unaffected. This fact may be partly accounted for by the weakening of the effect of breakages with distance (see above). The parts of the V-shaped autosomes adjacent to the locus of the spindle fiber (which is located in these chromosomes at the apices of the V's) are known to be much longer cytologically than suggested by the genetic maps (Dobzhansky, 1930*a*, 1930*b*). This discrepancy of the genetic and

the cytological maps is apparently due to the lower frequency of crossing-over per unit of the absolute distance in the vicinity of the spindle fiber as compared with the frequency of crossing-over in parts remote from the spindle fiber. Therefore, the effect of breakages on crossing-over must seem to become more rapidly weakened toward the spindle fiber, and less rapidly toward the free end of the chromosome. It may be questioned, however, whether the observed independence of the two limbs of the V-shaped autosomes can be completely accounted for by the factor just mentioned. Some facts seem to point in a different direction, suggesting that the homologous loci of the spindle fiber attachments possess a stronger attraction toward each other than other loci in the chromosomes. This point must be decided by further investigations. It must, however, be taken into account that the observed facts can not be explained by attraction of the loci of the spindle fibers alone (see above).

The value of the attraction hypothesis consists, primarily, in the fact that it can be tested experimentally. One such test is provided by the study of the interaction between the translocations and the inversions. Let us suppose that the chromosome B^1 (Fig. 1) carries an inverted section. In this case the attraction between the chromosomes B^1 and B^2 is weakened. Hence, the conjugation of the chromosomes A^1, A^2 and A^3 may proceed with relatively little interference of forces pulling B^2 toward B^1, and the frequency of crossing-over in A must rise. As shown above, this is found to be the case in translocations involving chromosomes II and III (Sturtevant and Dobzhansky).

A similar test is arranged in the following way. Suppose, for example, the chromosome A^1 (Fig. 1) carried an inverted section located within the part of the chromosome A^1 which is homologous to A^3. In such an event the attraction between A^1 and A^3 would be weakened. Therefore, the pairing of the chromosome A^2 with A^1

would be no longer interfered with, and the frequency of crossing-over in A^2 must rise. An experiment conforming to the scheme just outlined was arranged, and gave the expected result.

A situation may arise when the attraction of the fragment A^3 to its homologue A^1 is so strong that it may overpower the attraction of the recipient chromosome B^2 to its homologue B^1. Such a situation may be produced if the length of A^3 (Fig. 1) is much greater than that of B^2, or if no chromosome completely homologous to B^2 is present in the cell. Translocations involving the second and Y-chromosomes seem to furnish an example of such a situation. It has been found (see above) that if a very long section of the second chromosome is attached to the Y-chromosome, the frequency of crossing-over in the second chromosome is normal or nearly so. If, however, a smaller section of the second chromosome is attached to the Y, the frequency of crossing-over in the second chromosome is decreased.

The supposition that the homologous loci in the chromosome attract each other at a certain moment of gametogenesis is by no means a new one. It is a well-known fact that the homologous chromosomes may lie far apart from each other during the premeiotic and the leptotene stages of meiosis. The chromosomes, however, shift toward each other, and during the pachytene stage appear as closely associated threads. It is known furthermore that the chromomeres which lie side by side in the pachytene stage are always of the same size and shape, while chromomeres following each other in a linear series may be of different size. These observations suggest so much the existence of attraction between homologous chromomeres that they were actually described in such terms by many cytologists. The works of Belling (1928), Darlington (1929), Gelei (1922), Metz (1925), Metz and Nonidez (1921), Reuter (1930) and Robertson (1930) may be referred to here. Most of the cytologists, however, use the expression "attraction" in

a merely descriptive sense; this is obvious from the fact that the expressions "attraction" and "affinity" are used indiscriminately by some authors.

The experimental evidence adduced in the present paper seems to furnish, however, a genetical counterpart to the cytological observations leading to the assumption of the existence of an attraction between the homologous loci in the chromosomes. It may be supposed that a physical force attracting homologous loci to each other at meiosis actually exists. The phenomena of translocation and inversion furnish a method of attack on the problems here involved. What is the nature of the force which attracts the homologous genes to each other is completely unknown at present. In most objects (except in those which manifest the so-called somatic pairing of chromosomes) this force is active only during the meiotic stages, but not at other times. Any speculations concerning the nature of these forces seem to be unprofitable at present.

The present work is based on the study of various translocations in *Drosophila,* which were carried on in years 1928–1930 at the California Institute of Technology. The author takes the opportunity to acknowledge his obligations to Dr. T. H. Morgan and to Dr. A. H. Sturtevant for their valuable suggestions and criticisms. Best thanks are due to Dr. Sturtevant in closest contact with whom the work was done.

LITERATURE CITED

Anderson, E. G.

1929. "Studies on a Case of High Non-disjunction in *Drosophila melanogaster*," *Zeits. ind. Abst. Vererb.*, 51: 397–441.

Belling, J.

1927. "The Attachment of Chromosomes at the Reduction Division in Flowering Plants," *Jour. Genetics,* 18: 177–205.

1928. "The Ultimate Chromosomes of Lilium and Aloe with Regard to the Number of the Genes," Univ. California publ. Botany, 14: 307–318.

Belling, J., and A. F. Blakeslee.
1926. "On the Attachment of Non-homologous Chromosomes at the Reduction Division in Certain 25-chromosome Daturas," *Proc. Nat. Acad. Sci.*, 12: 7–11.

Blakeslee, A. F.
1927a. "Nubbin, a Compound Chromosomal Type in Datura," *Ann. New York Acad Sci.*, 30: 1–29.
1927b. "Genetics of Datura," Verhand. V internat. Kongr. Vererb. (*Zeits. ind. Abst. Vererb.*) *Supplement* 1: 117–130.
1929. "Cryptic Types in Datura," *Jour. Heredity*, 20: 177–190.

Blakeslee, A. F., and R. E. Cleland.
1930. "Circle Formation in Datura and Oenothera," *Proc. Nat. Acad. Sci.*, 16: 177–183.

Bridges, C. B.
1923. "The Translocation of a Section of Chromosome II upon Chromosome III in Drosophila," *Anat. Record*, 24: 426–427.

Burnham, C. R.
1930. "Genetical and Cytological Studies of Semisterility and Related Phenomena in Maize," *Proc. Nat. Acad. Sci.*, 16: 269–277.

Cleland, R. E., and A. F. Blakeslee.
1930. "Interaction between Complexes as Evidence for Segmental Interchange in Oenothera," *Proc. Nat. Acad. Sci.*, 16: 177–183.

Darlington, C. D.
1929. "Ring Formation in Oenothera and Other Genera," *Jour. Genetics*, 20: 345–363.
1929b. "Polyploids and Polyploidy," *Nature*, July: 15 pp.

Dobzhansky, Th.
1929. "A Homozygous Translocation in *Drosophila Melanogaster*," *Proc. Nat. Acad. Sci.*, 15: 633–638.
1930a. "Translocations Involving the Third and Fourth Chromosomes of *Drosophila Melanogaster*," *Genetics*, 15: 347–399.
1930b. "Cytological Map of the Second Chromosome of *Drosophila Melanogaster*," *Biol. Zentralblatt*, 50: 671–685.

Gelei, J.
1922. "Die Konjugationsfrage der Chromosomen in der Literatur und meine Befunde," *Arch. Zellfors.*, 16: 299–370.

Hamlett, G. W. D.
1926. "The Linkage Disturbance Involved in the Chromosome Translocation I of Drosophila, and Its Probable Significance," *Biol. Bulletin*, 51: 435–442.

Håkansson, A.
1929. "Chromosomenringen in Pisum und ihre mutmassliche genetische Bedeutung," *Hereditas*, 12: 1–10.

Metz, C. W.
1925. "Prophase Chromosome Behavior in Triploid Individuals of *Drosophila Melanogaster*," *Genetics*, 10: 345–350.

Metz, C. W., and J. F. Nonidez.
1921. "Spermatogenesis in the Fly, *Asilus sericeus* Say.," *Jour. Exper. Zoology*, 32: 165–185.

Meurman, O.
1929. "Association and Types of Chromosomes in *Aucuba japonica*," *Hereditas*, 12: 179–209.

Muller, H. J.
1928a. "The Problem of Genic Modification." Verhand. V. internat. Kongr. Vereb. (*Zeits. ind. Abst. Vererb.*) *Supplement* I: 234–300.
1928b. "The Production of Mutations by X-rays," *Proc. Nat. Acad. Sci.*, 14: 714–726.
1930. "Oenothera-like Linkage of Chromosomes in Drosophila," *Genetics*, 22: 335–357.

Muller, H. J., and E. Altenburg.
1930. "The Frequency of Translocations Produced by X-rays in Drosophila," *Genetics*, 15: 283–311.

Oliver, C. P.
1930. "The Effect of Varying the Duration of X-ray Treatment upon the Frequency of Mutations," *Science*, 71: 44–46.

Painter, T. S., and H. J. Muller.
1929. "The Parallel Cytology and Genetics of Induced Translocations and Deletions in Drosophila," *Jour. Heredity*, 20: 287–298.

Patterson, J. T., and H. J. Muller.
1930. "Are 'Progressive' Mutations Produced by X-rays?" *Genetics*, 15: 495–577.

Reuter, E.
1930. "Beiträge zu einer einheitlichen Auffassung gewisser Chromosomenfragen," Acta Zoologica Fennica, 9: 1–487.

Robertson, W. R. B.
1930. "Diploidy and Persistent Chromosome Relations in Parthenoproduced Tettigidae (Apotettix eurycephalus and Paratettix texanus)," *Jour. Morph. Physiol.*, 50: 209–257.

Sturtevant, A. H.
1921. "A Case of Rearrangement of Genes in Drosophila," *Proc. Nat. Acad. Sci.*, 7: 235–237.
1926. "A Crossover Reducer in *Drosophila melanogaster* due to Inversion of a Section of the Third Chromosome," *Biol. Zentralblatt*, 46: 697–702.

Sturtevant, A. H., and Th. Dobzhansky.
1930. "Reciprocal Translocations in Drosophila and Their Bearing on Oenothera Cytology and Genetics," *Proc. Nat. Acad. Sci.*, 16: 533–536.

SOME ATTEMPTS TO OBTAIN HAPLOIDS FROM *OENOTHERA LAMARCKIANA*[1]

PROFESSOR BRADLEY MOORE DAVIS

UNIVERSITY OF MICHIGAN

Oenothera Lamarckiana is believed to be a heterozygous species, the product of the union of gametes of different genetical constitutions, *gaudens* and *velans,* reproducing itself because in the main these are the only gametes formed which live and which give viable zygotes. If eggs of *Lamarckiana* could be induced to develop parthenogenetically and if the resultant haploids should be in some degree self-fertile it might be possible to obtain homozygous diploids which would help to solve the problem of the origin of *Lamarckiana.* With this in mind the writer in 1929 started some experiments which were concluded in 1930. The results, although negative, seem worthy of this report.

The chances were greatly against the success of the experiments for the reason that the gametes, *gaudens* and *velans,* carry lethals which will not allow the development of the zygotes *gaudens* · *gaudens* and *velans* · *velans,* these zygotes being represented by the more than 50 per cent. of sterile seeds found in *Lamarckiana.* It is to be expected that such lethals also would prevent the parthenogenetic development of *Lamarckiana* eggs although when brought together in the combination *gaudens* · *velans* their lethal powers are rendered ineffective. Nevertheless, there is the possibility that such lethals may at times be less harmful and it is even possible that lethals may become eliminated by gene mutations. In the event of such behavior the development of a haploid might be possible. For these reasons we may still hope that some one will be more fortunate and through experimental

[1] "Genetical Studies on Oenothera XVI." Papers from the Department of Botany, University of Michigan, No. 350.

treatment or through crosses obtain from *Lamarckiana* fertile haploids.

Since Haberlandt reports that he has induced early stages of parthenogenetic embryos in *Lamarckiana* by pricking and pinching ovaries, this procedure was practiced over a period of four weeks on plants of *Lamarckiana* from de Vries's line and on plants of a line derived from wild *Lamarckiana* growing at St. Anne's, England. After cutting the styles of mature flowers before pollination, ovaries on the main stem were in some cases pricked with a needle and in other cases slashed with a razor blade one or more times. The treated plants were not allowed to set any fruit on side branches, thus giving the main stem the benefit of all food produced. There were 102 ovaries thus treated on the *Lamarckiana* of de Vries's line and 98 ovaries on plants of the line from St. Anne's. All of these ovaries withered and finally fell off from the mother plants.

The other line of experimentation involved the pollination of *Lamarckiana* from a number of forms, some of them wide crosses, in the hope that these pollinations might stimulate the parthenogenetic development of some eggs either in company with the production of hybrid embryos or in connection with parthenocarpy. The pollinations were made from the following species and I am greatly indebted to Professor Bartlett, who assembled the material at the Botanical Garden of the University of Michigan and identified it.

Hartmannia speciosa (Nuttall) Small. Reading, England.

Megapterium missouriense (Simms) Spach. Vaughan's seed store.

Raimannia odorata (*Oenothera odorata* Jacq.). Hort. Bot. Conimbrigensis, Portugal.

Raimannia Sellowii (Link and Otto) Dresden, Germany.

Raimannia Drummondii (Hooker) Rose var. *nana* Hort. Reading, England.

Oenothera argillicola MacKensie. White Sulphur Springs, West Virginia.

Godetia amoena (Lehm.) G. Don. Leiden, Holland.

Godetia purpurea G. Don. Lund, Sweden.

Lavauxia acaulis (Cav.) Spach. Leiden, Holland.

Lavauxia acaulis (Cav.) Spach forma *alba* Hort. Reading, England.

Kneiffia Youngii Hort., a variety of *Kneiffia fruticosa* (L.) Raimann. Vaughan's seed store.

Kneiffia Fraseri (Pursh) Spach. Vaughan's seed store.

All seed-like structures obtained from the pollinations, after soaking in water for a few hours, were subjected to that alternate pressure and exhaust which appears to force water through the seed coats and starts the immediate germination on wet filter paper in Petri dishes of all viable seeds. Plants of *Lamarckiana* and of *lata* appeared in some of the cultures. These were undoubtedly accidental selfings, for the work was done with buds just ready to open in order that the stigmas might be in favorable condition for the germination of foreign pollen and with the large number of pollinations made under this condition some selfing could hardly be avoided.

The results of the pollinations are given in the accompanying tables and fall into three groups: *First,* pollina-

TABLE I

POLLINATIONS OF LAMARCKIANA WHICH YIELDED NO SEEDS OR CAPSULES

Cross	Pollinations	Capsule set
Lamarckiana (de Vries) × *Godetia amoena*	18	0
Lamarckiana (St. Anne's) × *Godetia amoena*	16	0
Lamarckiana (de Vries) × *Godetia purpurea*	5	0
Lamarckiana (de Vries) × *Lavauxia acaulis*	14	0
Lamarckiana (St. Anne's) × *Lavauxia acaulis*	18	0
Lamarckiana (de Vries) × *Lavauxia acaulis f. alba*	16	0
Lamarckiana (de Vries) × *Kneiffia Youngii*	16	0
Lamarckiana (St. Anne's) × *Kneiffia Youngii*	10	0
Lamarckiana (de Vries) × *Kneiffia Fraseri*	16	0

tions from the species *Godetia amoena, G. purpurea, Lavauxia acaulis* and its form *alba, Kneiffia Youngii,* and *K. Fraseri* yielded no seed and set no capsules (Table I.). *Second,* pollinations from *Hartmannia speciosa* and *Megapterium missouriense* set seed-like structures which proved to be sterile, that is, contained no embryos as determined by examination following unsuccessful attempts to germinate them (Table II). *Third,* pollinations from *Raimannia odorata* (*Oenothera odorata* Jacq.), *R. Sellowii, R. Drummondi* var. *nana,* and *Oenothera argillicola* gave fertile seed in various proportions from which came obvious hybrids accompanied in some cases by occasional dwarfs that failed to mature; there were no forms suggestive of a haploid *Lamarckiana* (Table II).

During the past two years there have been four independent reports on haploid oenotheras. Gates (1929) announced the appearance of a haploid from the cross *O. rubricalyx* x *O. eriensis.* This plant at maturity had the appearance of a miniature *rubricalyx* but was almost completely pollen sterile, and the few seeds obtained by selfing failed to germinate. Gates and Goodwin (1930) give an interesting comparison of nuclear and cell measurements from various tissues of this haploid in comparison with those of the diploid *rubricalyx.*

Stomps (1930*a*) has obtained sterile *Hookeri* haploids from the crosses *Hookeri* x *longiflora* and *Hookeri* x *argillicola.* The cross *franciscana* x *longiflora* gave a *franciscana* haploid sufficiently fertile to make selfing possible. Seeds of the selfed haploid produced typical *franciscana.* The haploid pollinated by *franciscana* likewise gave *franciscana* but in addition several forms interpreted by Stomps as similar to the trisomic *scintillans* and *lata* and to the triploid *semigigas.* Stomps (1930*b*) also reports *argillicola* haploids from the cross *argillicola* x *biennis* in the high proportion of 16 haploids to 41 hybrids, but the chromosome count in these plants was not established. This seems the more remarkable

TABLE II

POLLINATIONS OF LAMARCKIANA WHICH YIELDED SEEDS OR SEED-LIKE STRUCTURES

Cross	Pollinations	Capsules set	Seeds	Sterile seeds	Description of culture	Culture
Lamarckiana (de Vries) × *Hartmannia speciosa*	14	10 small	209	204	5 plants of Lamarckiana	30.101
Lamarckiana (St. Anne's) × *Hartmannia speciosa*	13	5 small	73	73	No germination	30.110
Lamarckiana (de Vries × *Megapterium missouriense*	25	20 small	111	102	8 plants of Lamarckiana 1 plant of lata	30.102
Lamarckiana (de Vries) × *Raimannia odorata*	12	8 small	235	235	No germinations	30.103
Lamarckiana (St. Anne's) × *Raimannia odorata*	7	5 small	208	175	1 plant of Lamarckiana 1 hybrid 31 etiolated seedlings which died	30.111
Lamarckiana (de Vries) × *Raimannia Sellowii*	15	13 medium and small	539	515	24 tall, strong plants, evidently hybrids	30.104

TABLE II—(*Continued*)

Cross	Pollinations	Capsules set	Seeds	Sterile seeds	Description of culture	Culture
Lamarckiana (de Vries) × *Raimannia Sellowii*	15	14 medium and small	412	398	12 tall, strong plants, evidently hybrids 2 narrow leaved dwarfs	30.105
Lamarckiana (St. Anne's) × *Raimannia Sellowii*	14	14 small	758	410	348 seedlings but almost all were etiolated and died early 23 tall, strong plants, evidently hybrids	30.112
Lamarckiana (St. Anne's) × *Raimannia Sellowii*	16	12 medium and small	846	185	661 seedlings but almost all were etiolated and died early 2 plants of Lamarckiana 19 tall, strong plants, evidently hybrids	30.113
Lamarckiana (de Vries) × *Raimannia Drummondii* var. *nana*	17	17 medium	1775	1770	5 etiolated seedlings which died	30.106
Lamarckiana (de Vries) × *Raimannia Drummondii* var. *nana*	19	12 medium	806	780	26 etiolated seedlings which died	30.107

TABLE II—(*Continued*)

Cross	Pollinations	Capsules set	Seeds	Sterile seeds	Description of culture	Culture
Lamarckiana (St. Anne's) × *Raimannia Drummondii* var. *nana*	20	20 medium	1721	1280	441 etiolated seedlings many of which died early 239 weak, etiolated hybrids few of which matured	30.114
Lamarckiana (St. Anne's) × *Raimannia Drummondii* var. *nana*	14	14 medium	1977	1830	147 etiolated seedlings many of which died early 6 plants of Lamarckiana 62 weak, etiolated hybrids few of which matured	30.115
Lamarckiana (de Vries) × *Oenothera argillicola*	18	18 medium	2550	1310	1240 seedlings produced a culture of vigorous hybrids except for a few etiolated plants	30.108
Lamarckiana (de Vries) × *Oenothera argillicola*	16	14 medium	953	290	663 seedlings produced a culture of vigorous hybrids except for a few etiolated plants	30.109
Lamarckiana (St. Anne's) × *Oenothera argillicola*	15	15 medium	1305	249	1056 seedlings produced a culture of vigorous hybrids except for a few etiolated plants	30.116

since he did not obtain haploids from *argillicola* pollinated by *Hookeri, muricata, Lamarckiana* and *Lamarckiana-blandida.*

Emerson (1929) published, in 1930, a report on a haploid *franciscana* almost completely sterile from the cross *franciscana* x *franciscana-sulfurea,* and gives an account of anastomosing threads in the early stages of the spirem during meiosis and of twisted loops during the second contraction. It has been suggested by Cleland and others that similar conditions as found in diploid oenotheras offer a cytological basis for crossovers, but Emerson points out that this suggestion does not find support in the haploid where these stages are also found, although homologous chromosomes are not expected to be present. It may, however, in fairness be noted that the possibilities of crossing over at such periods of meiosis in the diploid are not excluded by the findings in the haploid.

Davis and Kulkarni (1930) have described the genetics and cytology of a haploid that has appeared four times since 1923 in a selfed line of *Oenothera franciscana* with a frequency of about 1:1000. Three generations of selfed haploids have also been grown consisting chiefly of *franciscana* but with the haploid again appearing together with a number of other forms. The haploid was also noted from the crosses *franciscana* x *franciscana-sulfurea, franciscana* x *franciscana-sulfurea* dwarf, and (*franciscana-sulfurea* dwarf x *franciscana*) x *franciscana.* These crosses are between closely related plants and are not to be thought of as involving a stimulus from the pollen of distant types. The haploid back-crossed to *franciscana* gave *franciscana* as was to be expected. The chromosome count of the haploid was established in 1928 from a plant that appeared in 1927 in the line of *franciscana* and an account of microsporogenesis was presented. Davis and Kulkarni (1930) also reported a sterile haploid plant that appeared in 1927 out of a line of *Oenothera*

Hookeri that included three generations and totaled 1,291 plants.

A line of *franciscana* was started in 1929 through seeds from the original haploid plant selfed in 1923. Such *franciscana* plants synthesized by the selfing of a haploid should be strictly homozygous and the behavior of such a line becomes a matter of interest. The plants themselves can not be distinguished from the wild *franciscana* of the original line which has been carried through fifteen generations. From this synthesized *franciscana* the first generation of 818 plants was grown in 1930 and proved to be uniform *franciscana* except for two haploids which flowered and one narrow-leaved dwarf rosette that died early. Thus the ratio of haploids in this line of synthesized *franciscana* starts out higher than that of the wild *franciscana.*

Other cases of parthenogenesis are known in *Oenothera* and are described by Stomps (1930*b*), but they concern the appearance of diploids from tetraploids and have not yet received the close cytological study which they deserve. Physiologically, such diploids would be expected to be more vigorous than haploids, and meiosis should not present so much irregularity in its procedure. They constitute a class quite apart from the haploids and seem likely to have peculiarities of their own.

It was suggested at the beginning of this paper that the chances of obtaining haploids from the heterozygous *Lamarckiana* may not be favorable because of the lethals carried by its gametes. *Oenothera franciscana* and *O. Hookeri* are in contrast homozygous species with all pairing chromosomes and with pollen almost wholly good and seeds more than 90 per cent. viable. Such species might be expected to give haploids more readily and they have been found independently by Stomps, Emerson, and Davis and Kulkarni. *Oenothera argillicola* from which Stomps reports numerous haploids following pollination by *biennis* is probably also homozygous since its pollen

in my examinations has been about 85 per cent. good and its seeds about 80 per cent. viable. *Oenothera rubricalyx,* which gave a haploid to Gates, has three pairs of chromosomes together with a circle of eight (Cleland), and its pollen is only 50 per cent. good and its seeds about 40 per cent. viable. These facts indicate that *rubricalyx* is heterozygous, a conclusion supported by its breeding behavior. If *rubricalyx* may give haploids it becomes a matter of interest to learn what may be the limitations to the production of haploids among the heterozygous species of Oenothera.

LITERATURE CITED

Davis, B. M., and Kulkarni, C. G.
1930. The Cytology and Genetics of a Haploid Sport from *Oenothera franciscana.* *Genetics,* 15: 55–80.

Emerson, S. H.
1929. The Reduction Division in a Haploid Oenothera. *La Cellule,* 39: 159–165.

Gates, R. R.
1929. A Haploid Oenothera. *Nature,* 124: 948.

Gates, R. R., and Goodwin, K. M.
1930. A New Haploid Oenothera with some Considerations on Haploidy in Plants and Animals. *Jour. Gen.,* 23: 123–156.

Stomps, T. J.
1930a. Ueber Parthenogenesis infolge Fremdbefruchtung. *Zeitsch. f. ind. Abst. u. Vererb.,* 54: 243–245.
1930b. Ueber Parthenogenetische Oenotheren. *Ber. deut. bot. Gesell.,* 48: 119–126.

A PRELIMINARY REPORT ON SOME CHROMOSOME ALTERATIONS BY X-RAYS IN *CREPIS*

M. NAVASHIN

TIMIRIAZEV BIOLOGICAL INSTITUTE, MOSCOW

ON the basis of extensive observations the following classification of the various types of chromosome alterations have been noted by the writer:

1. *Quantitations:* Processes leading only to change of the chromosome number without any alteration of the chromosomes themselves. Subdivisions of this class are:

(a) Summations, *i.e.*, addition or subtraction of entire haploid chromosome sets (polyploidy and haploidy);

(b) Combinations, *i.e.*, addition or subtraction of entire individual chromosomes (trisomy, polysomy, monosomy, etc.).

2. *Dislocations:* Rearrangement of chromosome material without any alteration in the total mass of the nucleus. Here should be classified various alterations known to occur spontaneously, such as attachment, duplication, translocation, fragmentation, etc.

3. *Transformations:* Alteration of the morphological characters of the chromosomes invariably connected with a change of the total mass of the nucleus, although often affecting different chromosomes independently.

Transformation is believed to have occurred in course of the evolution of the *Crepis* species, which in most cases markedly differ in the sizes and shapes of their individual chromosomes.

4. *Novations:* A presumed phenomenon of formation of the chromosomes *de novo*, which was called to account for some apparently otherwise inexplicable cases of appearance of new large "V-shaped" chromosome structures. As will be shown in the following, this last type should very probably be merged with dislocations.

While quantitations and dislocations were relatively often (in 1.0 and 0.1 per cent. of cases, respectively) found to occur spontaneously among individuals of the same species, the third type, *viz.*, transformation, never was met with in a pure species. There seemed to be a lack of connection between the individual chromosome variation and the transformational process or processes which brought about the *specific* differences of the chromosomes.

In order to investigate the nature of these individual chromosome variations, an attempt was made to increase the frequency of their occurrence by means of X-ray treatment. In the following are reported the first results of these experiments.

Grateful acknowledgments are made here to R. A. Golonsko, M.D., and to M. A. Vassilevsky, M.D., who kindly made possible access to the necessary apparatus and helped in administering the X-rays.

As material for this investigation *Crepis tectorum* was chosen because it has only four pairs of chromosomes, each being morphologically clearly distinguishable from the others; also because it was known to throw many viable chromosome aberrations. Seeds (achenes) of a certain strain (No. 579), after having been soaked for twenty hours on wet filter-paper in a Petri dish, were exposed to various dosages of X-rays. The conditions of the experiment were as follows:

Distance from the target	20 cm
Kilovolts	108
Milliamperes	4.0
Aluminum filter	.5 mm

Exposures were made in the following way. All the seeds (after the control group was removed) were placed under the tube and exposed for forty seconds. Then, after having disconnected the current, a portion of seed was removed and the remaining seeds exposed again, this time for eighty seconds. Then the current was disconnected again and the second portion of irradiated seeds was removed, after which the remaining material was exposed for one hundred and twenty seconds. Thus the treatment was continued until the tenth portion. The exposures thus rapidly increased in the form of a progression, the tenth portion of material being irradiated totally for 2,200 seconds.

The peculiarity of the treatments administered in the above manner consisted in their discontinuity, for the

dosages, beginning with the second portion of seed, consisted of 2, 3, 4—10 separate exposures; alternating with short periods of rest during which the subsequent portions of treated material were removed.

After having been treated in the above manner the seeds were planted in pots containing soil. All ten lots germinated in a normal way and the seedlings did not differ from those which came from the untreated (control) seed of the same strain. Very soon after the germination, however, the treated individuals displayed marked influences of X-rays. These influences appeared as early as six days after germination and consisted in delayed or abnormal development of the first leaves. The proportion of such abnormalities was clearly connected with the dosages employed. While the first portions, treated with shorter exposures, showed but few abnormalities, the last portions contained only very few apparently normal individuals. The tenth portion,

TABLE I

DATA ON THE DOSAGES OF X-RAYS ADMINISTERED TO DIFFERENT PORTIONS OF SEED OF *Crepis tectorum* AND ON THE GERMINATION AND FIRST STAGE OF DEVELOPMENT OF THE SEEDLINGS

Portion number	Culture numbers	Exposure in seconds	Number of seeds	Number germinated	Died in early stages	Appearance of the young plants
0 (control)	30 × 1–0	.....	10	9	.....	
1	30 × 1–1	40	11	11	.....	Occasional abnormalities
2	30 × 1–2	120	10	8	.....	" "
3	30 × 1–3	240	10	7	.....	" "
4	30 × 1–4	400	10	9	.....	" "
5	30 × 1–5	600	10	9	.....	" "
6	30 × 1–6	840	10	8	.....	" "
7	30 × 1–7	1,120	10	8	.....	" "
8	30 × 1–8	1,440	11	11	3	Many abnormal
9	30 × 1–9	1,800	10	10	3	The majority abnormal
10	30 × 1–10	2,200	36	26	13	All abnormal

treated altogether for 2,200 seconds, consisted only of abnormal individuals, a considerable part of which died prior to forming the first leaf. The above data are tabulated in Table I.

In the following will be given only a short account of the observations made on somatic chromosomes in the root tips taken from the plants belonging to the last three groups of this experiment. All other data on the treated plans as well as on reduction phenomena and on other experiments performed will be published elsewhere.

The cytological examination of root tips taken from the plants nearly three months old revealed an extraordinarily high proportion of altered individuals. The average percentage of these was as high as 60 for the three groups of plants, while the last group (exposed for 2,200 seconds) contained no normal individuals at all. It thus appeared that the X-ray treatment employed increased the "mutation" rate about 600 times on the average (the natural rate of spontaneous dislocations being *ca* 0.1 per cent., as was noted above).

All the chromosome alterations induced by the treatments represented the result of dislocations. No single case of quantitation was observed. The great majority of these alterations appeared in chimeral seedlings, the most extreme individuals producing roots of as many as six karyologically different types. In two cases, however, identical chromosome alterations were found to be present in all the roots subjected to cytological investigation. This seems to furnish evidence in favor of the theory that the whole root develops from a single initial cell.

The normal complement of *C. tectorum* consists of four pairs of chromosomes which differ one from another as to size, place of the kinetic constriction (place of spindle fiber attachment) and presence or absence of satellites (see Fig. 1, *i*). Since the constrictions are connected with the kinetic functions of the chromosomes, they are

oriented with respect to the center of the equatorial plate so that the longer arms of the chromosomes are directed more or less toward the periphery. The shorter arm of the chromosome consequently is always turned toward the center, its end being thus the "proximal" end of the chromosome and the longer arm terminating at the "distal" end. The "A-chromosomes" consist of two arms, one of which is approximately twice as long as the other, the kinetic constriction being consequently located between the second and the third thirds of the chromosome length, counting from the distal end. The "B-chromosomes" are somewhat longer than the A's but possess subterminal constrictions, thus being composed of arms one of which exceeds the other in length about 6 to 7 times. The "C-chromosomes" are considerably shorter than the B's, but their shorter arms are slightly longer than in the B's, the constriction being located somewhat farther from the proximal end. The "D-chromosomes" have highly reduced shorter arms which appear as very small round "knobs," the constriction being located very near to the proximal end. The D's possess in addition satellites, very small spherical appendixes connected with the chromosome knobs by thin threads.

The nature of the chromosome alterations induced by the treatments is shown in Fig. 1, representing somatic chromosomes from the root tips of several mutated individuals. One may see from this figure that the alterations always involve not less than two chromosomes, since a portion of one chromosome is detached and transferred to another homologous or non-homologous chromosome. Proximal fragments of various sizes become autonomous diminished chromosomes, whereas the distal fragments invariably appear fused with other chromosomes. The only reason for such behavior is found in the fact that the distal fragments possess no kinetic constrictions and are eliminated unless they associate with some other chromosome supplied with a constriction. It

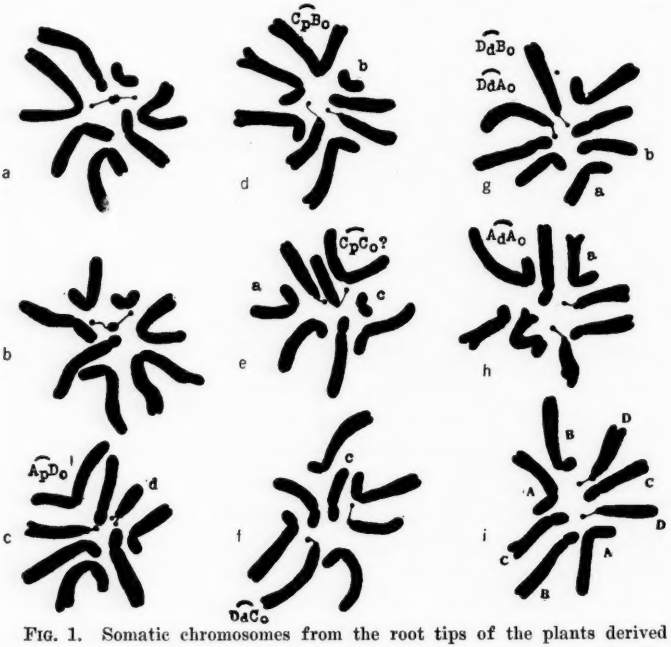

FIG. 1. Somatic chromosomes from the root tips of the plants derived from seeds exposed to X-rays for 1,440 to 2,200 secs.; *a*, *b*, very complex dislocations involving the majority of the chromosomes (the small bisatellited body being the product of fusion of the satellited fragments of the two D-chromosomes); *c*, $A_pD_o + d$; *d*, $C_pB_o + b$; *e*, $C_pC_o? + a + c$; *f*, $D_dC_o + e$; *g*, $D_dA_o + D_dB_o + a + b$; *h*, $A_dA_o + a$; *i*, normal diploid complement of *C. tectorum*.

In all cases small letters indicate free proximal fragments containing a kinetic constriction; capital letters with the subscript o, distal fragments deprived of constrictions; horizontal brackets, fusion; subscripts p and d, the proximal and the distal ends of the chromosome, respectively, where fusion takes place. Capital letters without subscripts indicate the four types of the normal chromosomes. In addition to the dislocations indicated above, there are probably minor alterations present in some cases (in Fig. 1 *d*, for instance, the smaller arm of the free B-chromosome appears to be somewhat enlarged). See also text, p. 247. Magnification ca. 1,700 diameters.

should be noted, further, that normal unaffected chromosomes were never observed to fuse together. On the contrary, fusion was invariably connected with fragmentation. This seems to indicate that both processes (*i.e.*,

fragmentation and fusion) are due to some common cause, most probably related to some sort of interchange between non-homologous chromosomes.

In order to make possible a brief description of the alterations observed, the following system of designation was used. A detached proximal fragment containing the kinetic constriction was designated by a corresponding small letter. To designate the distal fragment of the same chromosome the same capital letter was used with the subscript o. Thus, for example, the proximal and distal fragments of a D-chromosome were designated by "d" and "D_o," respectively. To indicate fusion, a horizontal parenthesis was used. Finally, to indicate the point of fusion (in case it could be clearly determined) subscripts p and d were applied, signifying the proximal and distal ends, respectively. Thus, for instance, the formula $A_pD_o + d$ (Fig. 1 *c*) signifies that the distal part of the D-chromosome had been transferred and attached to the proximal end of an unaltered A-chromosome, while the proximal fragment of the same D-chromosome became an autonomous small chromosome.

The particular instances of alterations of individual chromosomes are explained in the legend for Fig. 1.

From cases represented in Fig. 1, as well as from many others which were observed, it might be concluded that there seemed to be no limitations as to size of the dislocated chromosome fragments. Nor did there seem to be any regularity as to the direction of the process. On the contrary, in the comparatively small amount of material examined (altogether 26 plants) nearly all possible combinations were found, so that it could be deduced that probably parts of any chromosome, of any size, can be transferred to any other chromosome and attached either to the proximal or to the distal end of the same.

The kinetic constriction, on the other hand, exhibited an outstanding stability. No cases where a constriction disappeared or was formed on a new place were found

in these experiments.[1] Nor were chromosomes with more than one constriction formed. It seems thus very probable that the main reason for the stability of chromosome number in a given group of related organisms (when polyploidy is excluded) is the above noted high stability of the kinetic constrictions, the number of which always corresponds to the number of chromosomes. It is not meant by this that the constriction can not be destroyed or changed but merely that the chromosomes lose their vitality or their ability to function normally when the kinetic constrictions are damaged or altered in some fundamental way. Loss of the normal constriction results in elimination of chromosomes so affected or of their fragments, unless they become associated with some other chromosome having a constriction.

It should be noted in this connection that dislocation of the chromosome material is not associated in any way with the formation of chromosome constrictions. As was shown in Fig. 1, in no case could any trace of constriction be seen at the place where a detached fragment was fused with the end of another chromosome. Experimental evidence thus seems to contradict the hypothesis that constrictions represent places of end-to-end fusion which might have taken place in the course of evolution. As for the question whether the dislocations represent only rearrangements of chromosome material or whether they can lead also to gain or loss, nothing definite can be said at the present time. Judging from what is known for other organisms, it seems rather probable, however, that cases of the latter sort should be not infrequent. At any rate, it is clear that quantitative variation in the total chromatin material would necessarily arise in the progeny of altered individuals due to recombinations of dislocated chromosomes. And, if viable, some of these descendants may obtain chromosome comple-

[1] The only case of *de novo* formation of the constriction in the distal fragment of the fragmented D-chromosome in *C. tectorum* was established by the writer in 1926.

ments transformed in the way which is characteristic of many related species.

Irrespective of their kind, the observed chromosome alterations did not seem to affect in any way the vitality of the cells or organs involved. Nor did even the most conspicuous dislocations (such as those represented in the Fig. 1 *a* and *b*) seem to influence seriously the growth or the size of the roots. It was thus evident that the various dislocations involving very different quantities and qualities of the chromatin material did not produce any "positional effect." On the contrary (at least in the presence of some unaltered chromosomes), all the roots, the cells of which were affected in different ways by chromosome dislocations, developed in an apparently normal manner.

As was shown above, the products of dislocations induced by X-ray treatment often assume a form of large "V-shaped" (two-armed) chromosomes usually composed of products of fragmentation fused with normal chromosomes. As was also stated, similar structures have been observed to occur spontaneously, in some cases even in association with obvious chromosome fragmentation. Due to some complications (loss of fragments, presence of extra chromosomes, etc.), the first observations on these spontaneous dislocations led the writer to an erroneous interpretation of the phenomenon in question. It was deduced, namely, that the occurrence of the new "V-shaped" chromosomes must be attributed to some peculiar process of *de novo* formation ("Novation"). At the present time when the experiment here reported produced essentially the same products through dislocations, there can be hardly any doubt that "Novation" represents nothing but dislocation involving large chromosome fragments.

It is of interest to note in this connection that the frequent formation of the V-shaped chromosomes through dislocation makes it possible that such chromosome

shapes occurring naturally, as they do in a great many species, may represent secondary structures of more recent origin. If such is the case, it would seem to contradict the hypothesis advanced by some writers that the chromosomes were primarily V-shaped. Such evidence, however, must be considered in connection with all other available evidence on the phylogeny of groups of species.

It is premature as yet to draw definite conclusions from the above data. It may be suggested, however, that dislocations should play a decisive rôle in species formation. Firstly, because they cause reorganization of the linkage relations which in most cases must inevitably result in a certain degree of cross sterility and even in practically complete physiological isolation due to altered affinity and disturbed conjugation of the dislocated chromosomes. Secondly, because dislocations may lead also, under certain circumstances, to chromosome transformation such as that postulated in the explanation of the evolution of chromosome structure. As a matter of fact, it is easy to see that subtraction or addition of varying amounts of chromatin material, differing in quality in different chromosomes and at different levels of individual chromosomes, opens up vast possibilities for genetic variation which would furnish material for the operation of natural selection and other evolutionary forces. Further investigations on the progeny derived from the treated individuals are in progress and new experiments are under way. Particular attention will be devoted to the obtaining of balanced (homozygous) types possessing pairs of identically dislocated chromosomes: to experiments bearing upon the problem of the time when induced chromosome alterations take place, and on the mode of their occurrence.

INHERITANCE AND LINKAGE RELATIONS OF CHOCOLATE PERICARP IN MAIZE[1]

DR. E. G. ANDERSON
CALIFORNIA INSTITUTE OF TECHNOLOGY
AND
PROFESSOR R. A. EMERSON
CORNELL UNIVERSITY

SOURCE OF MATERIAL

AN open pollinated ear of chocolate maize was obtained from Mr. F. D. Richey, of the U. S. Department of Agriculture. The source of this ear was unknown, but the growth habit and plant color (weak purples and weak sun reds) of its progeny suggest a South American origin. Linkage tests have been made with all the known linkage groups using chocolate from this source.

During 1924 seed of chocolate maize was collected by Richey and Emerson at Sicuani, Peru. This chocolate was crossed with an early corn (New Brunswick flint) obtained from Mr. Richey, and has also been tested with all the known linkage groups. While the identity of the character from the two sources has not been completely established, the similarity in appearance and the failure of both stocks to show linkage with any of the nine known linkage groups indicate that the same gene is involved.

DESCRIPTION

Chocolate pericarp color is a simple dominant to colorless. The pericarp develops a dull grayish brown to chocolate pigmentation unlike any of the pigments commonly found in maize. A typical chocolate pericarp over white endosperm, compared with Ridgeway's "Color Standards and Nomenclature," approximates the color Drab 17^{1111}. The cob also is colored. This is most clearly shown when the cob is broken. When

[1] Paper No. 176, Department of Plant Breeding, Cornell University, Ithaca, New York.

homozygous, the pigmentation is usually somewhat more intense than when heterozygous. The symbol *Ch* is suggested for the chocolate gene.

There is ordinarily no difficulty in classifying chocolate in mature ears except in the presence of self red, brown or cherry pericarp color, and even very immature ears show the color on drying. Purple and dilute purple plants occasionally show enough purple color in the pericarp to interfere. With homozygous purple aleurone identification of chocolate pericarp is difficult though

TABLE I

SUMMARY OF LINKAGE TESTS INVOLVING CHOCOLATE PERICARP COLOR

Genes	Phase*	XY	Xy	xY	xy	Total	Recombinations	Per cent. of recombinations
Ch–C	C	240	254	281	283	1058	535	50.6
Ch–Sh	C	17	5	27	10	59	32	54.2
Ch–Wx	C	31	6	34	9	80	40	50.0
Ch–R	C	163	185	174	202	724	359	49.6
"	R	203	172	209	177	761	380	49.9
Ch–G	C	72	45	55	42	214	100	46.7
Ch–Su	C	422	382	442	400	1646	824	50.1
Ch–Tu	R	28	30	20	24	102	52	51.0
Ch–B	C	837	858	867	833	3395	1725	50.8
"	R	175	175	170	172	692	347	50.1
Ch–Lg	C	146	151	143	151	591	294	49.7
"	R	42	53	52	52	199	94	47.2
Ch–Pl	C	159	169	162	152	642	331	51.6
"	R	314	329	316	332	1291	646	50.0
Ch–Y	R	279	288	319	313	1199	592	49.4
Ch–Ts$_2$	C	122	118	124	120	484	242	50.0
Ch–F	C	10	11	9	13	43	20	46.5
Ch–Ra	C	105	83	102	88	378	198	52.4
Ch–A	C	387	425	425	413	1650	850	51.5
"	R	38	57	49	50	194	88	45.4
Ch–Ts$_1$	C	59	80	70	68	277	150	54.2
Ch–D$_1$	C	50	25	45	13	133	70	52.6
Ch–Cr	C	50	25	43	15	133	68	51.1
Ch–Pr	C	191	182	200	207	780	382	49.0

* C = coupling and R = repulsion.

usually not impossible. In such cases cob color is helpful in the classification.

The development of chocolate pigmentation appears to be independent of the anthocyanin flavonol pigment system inclusive of the red and brown pigments of the pericarp. It develops uniformly on all plant color types. It also develops in the presence of other pericarp colors, though it may be obscured by them. Ears having the genes for red variegation and for chocolate show red stripes on a chocolate ground.

Linkage Tests

A summary of linkage tests involving chocolate pericarp color is presented in Table I. These tests involve one or more genes from each of the known linkage groups. There is no indication of linkage with any of these nine groups. Some of these tests include two genes of the same group simultaneously. These tests are summarized in Table II. In addition F_2 data have been obtained from crosses of *Ch* with gl_1, v_5 and v_2. In no case is there any indication of linkage.

Discussion

The linkage tests reported indicate that the chocolate gene does not belong to any of the nine known linkage groups. It may therefore represent the remaining tenth group. But since crossing-over occurs freely in both megasporogenesis and microsporogenesis, linkage tests alone can not with certainty exclude a gene from any linkage group. It always remains possible that the known linkage maps do not cover a sufficient portion of the chromosome and that, therefore, apparent independence may only mean that the gene in question lies at some distance from the known or tested portion of the map. This uncertainty is illustrated by the recent results of Brink and Senn (in press) and of Holmeyr (unpublished) in which d_1 and *cr* are united in one group with *a* ts_4 and *na* through the intermediate genes *Rg*

TABLE II

LINKAGE TESTS INVOLVING TWO KNOWN GENES IN THE SAME CHROMOSOME

Order of genes	Crossover regions			
	0	1	2	1–2
Ch / *sh wx* / *Ch*	34	6*	32	8*
sh wx / *Ch*	34	32	8*	6*
su Tu / *Ch*	37	15	32	20
su Tu / *Ch*	37	32	20	15
B / *lg Ch*	72	33	63	31
B / *Ch lg*	72	63	31	33
Pl Ch / *Y*	186	66	206	80
Ch Pl / *Y*	186	206	80	66
Ch / *a* ts_4 / *Ch*	62	65	72	78
a ts_4 / *Ch*	62	72	78	65
d_1 *cr* / *Ch*	55	10	60	8
d_1 *cr*	55	60	8	10

* Germination of shrunken seeds was poor.

(ragged) and ba_1 (barren stalk), respectively, corroborated by McClintock by means of trisomic ratios involving *a* and *cr*.

The independence of the majority of the known groups has been established by means of aberrant endosperm (Emerson, 1924) or by trisomic inheritance (McClintock and Hill, 1931). Both of these methods are independent of map distance and should therefore be decisive. It is hoped that the tenth group represented by chocolate may be subjected to similar rigid tests in the near future.

Résumé

Chocolate pericarp color in maize is a simple dominant to colorless. Tests with the nine known linkage groups give no indication of linkage. The gene *Ch* is therefore submitted as representing the tenth linkage group.

LITERATURE CITED

Brink, R. A. and Senn, P. H.

1931. "Ragged, a Dominant Character in Maize Linked with A_1 Ts_4 and D_1," *J. Heredity* (in press).

Emerson, R. A.

1924. "Aberrant Endosperm Development as a Means of Distinguishing Linkage Groups in Maize," AMERICAN NATURALIST, 58: 272–277.

Hofmeyr, J. D. J.

"The Inheritance and Linkage Relations of Barrenstalk-1 and Barrenstalk-2, Two Mature Plant Characters of Maize" (unpublished thesis).

McClintock, Barbara and Hill, Henry E.

1931. "The Cytological Identification of the Chromosome Associated with the R. G. Linkage Group in *Zea mays*," *Genetics* (in press).

ORIGIN OF MAMMALIAN FAUNAS AS ILLUSTRATED BY THAT OF FLORIDA

DR. GEORGE GAYLORD SIMPSON

AMERICAN MUSEUM OF NATURAL HISTORY

THE study of evolution as a sequence of historic events, a fundamental aspect sometimes ignored by the biologist, is concerned with three things: origin, or change in structure; migration, or change in geographic distribution, and extinction. The underlying causes of these changes, the field of the evolutionary biologist, must be considered in the light of a knowledge of what the changes actually were, the field of the paleontologist. To consider what can happen, or how changes may occur, without reference to what actually has happened, is to divorce the science from reality.

In seeking to account for the origin of living animals there are numerous possible approaches. Many of these, including some of the most valuable, deal with isolated animals or single processes, or go to the opposite extreme of dealing with abstract principles without respect to actual causes. These may be checked and coordinated by the use of a method which has not, I believe, been fully expressed or appreciated. The data for this method would consist of an exact knowledge of successive animal societies, faunas, in a single region. The interpretation of these data would involve an elucidation of the differences and resemblances between these faunas. The ultimate aim would be the discovery of the causes for these relationships.

It is the present purpose to give an example of this approach toward the general problem of evolution. The facts are presented so far as they are known. The interpretation of these facts is certainly incomplete and probably erroneous in part, but the facts themselves are

concrete data on which theories may be based or against which they may be checked.

The example chosen consists of successive faunas, Pleistocene and Recent, of a limited area, the State of Florida. This example is taken because the faunas are fairly well known, much better than most faunas, and because the area is one naturally delimited and of especial intrinsic interest. Attention is directed to the land mammals, because these are by far the best known and because they do constitute a natural faunal group.

Origin and Composition of the Pleistocene Fauna

The best-known fossil fauna from Florida is that of the so-called Melbourne beds, and attention will be confined to species that are known to be derived from deposits of this age. From the circumstance that it contains most of the living species of Florida, it is probable that this fauna is of late Pleistocene age. Some authorities consider it as considerably older, but that is not important from the present point of view. It is a fauna of contemporaneous land mammals which preceded the recent fauna, by however long a period.

As now known to me, and after careful revision, this Melbourne fauna includes at least sixty-six species of land mammals; distributed ordinally as follows:

Marsupialia	1	Edentata	7
Insectivora	3	Perissodactyla	6
Rodentia	17	Artiodactyla	11
Carnivora	17	Proboscidea	4

This represents unusually complete knowledge for an extinct fauna, yet it doubtless does not include all the species then present here. It is probable that there were at least ten more species, a majority of which were probably small rodents and most of the rest probably carnivores.

It is reasonable to estimate that at least seventy-five species were present, of which at least twenty were rodents and almost as many carnivores. For the most

part, however, analysis may be based on what is known, with this suggestion as to the probable nature of the undiscovered part of the fauna.

The ultimate origin of this fauna is fairly well known. The species are all exclusively North American, and a majority of them were probably confined to the east or southeast. Ten species (edentates and three rodents), about 15 per cent. of the known fauna, belong to groups introduced from South America in the Pliocene or early Pleistocene. Nine species (among rodents, artiodactyls and proboscideans), about 14 per cent., belong to groups probably introduced from Eurasia not earlier than the late Pliocene, and perhaps some others belong here. The rest, about 70 per cent., appear to be old residents or truly autochthonous. This mingling of mammals of at least three very distinct geographic origins is an essential and striking factor in the Pleistocene fauna.

ORIGIN AND COMPOSITION OF THE RECENT FAUNA

In considering the recent fauna it is proposed to ignore subspecies and to include only land mammals probably inhabiting continental Florida in early historic times. The fragmentary nature of the materials and the lack of distributed geographic samples make determination of subspecies (in the sense of recent zoology) almost impossible to the paleontologist, and if they were considered the faunas would not be comparable.

On this basis, using some personal judgment as to the species recognized, there were thirty-three land mammals in the late prehistoric fauna of Florida, ordinally distributed as follows:

Marsupialia	1	Carnivora	11
Insectivora	3	Artiodactyla	1
Rodentia	17		

Twenty-three of these species also occur in the Pleistocene. Their immediate origin is obvious. The ten spe-

cies which are not known from the Pleistocene are as follows:

Sciurus niger (Fox squirrel).
Glaucomys volans (Flying squirrel).
Peromyscus (four species) (White-footed mice).
Pitymys parvulus (Pine mouse).
Mustela vison (Mink).
Canis floridanus (Wolf).
Felis coryi (Cougar).

The rodents are all small animals, some indeed minute, and would be little likely to be preserved as fossils or, if preserved, to be found. They may well have been present in the Pleistocene. Their wide distribution and local differentiation in the state suggests that they are old residents. The apparent absence of the mink in the Pleistocene may also be accidental, as it is a small animal and one which was probably not common. The weasel is known from only two or three fossil specimens.

The absence of the two large modern predaceous carnivores in the Pleistocene may be more significant. If *Canis floridanus* is correctly distinguished from *Canis lycaon,* this fact in itself might stamp it as a fairly old inhabitant. Large canids are, however, rather common in the Pleistocene, and so far as determinable they appear to belong without exception to the extinct subgenus *Ænocyon,* the so-called dire wolves. Some of the indeterminate remains may represent the true wolves, or these may have been present but not discovered as yet. It seems clear that dire wolves were predominant among large Pleistocene canids and that true wolves were relatively few or may possibly, but improbably, not have entered Florida until Recent time.

Felis coryi, the other large predaceous carnivore of Recent Florida, is also unrecorded in the Pleistocene. It also is said to be a species peculiar to Florida, and may be an old inhabitant. Cougars do occur in the Pleistocene, close relatives of *F. coryi,* and it is possible that some of them have been incorrectly distinguished from that species. The question remains open.

About two thirds of the present fauna is known to have been derived directly from the Pleistocene fauna of the same region. Of the remaining third it is possible that one or two species are post-Pleistocene immigrants, but it is probable that all were derived from Pleistocene inhabitants.

In most or all of such cases as permit close comparison, the recent species are identical with their Melbourne age forerunners. Post-Melbourne evolution has on the whole been of subspecific or lesser value.

The ultimate origin of the Recent fauna is less complex than that of the Pleistocene fauna. No species belong to South American groups, and perhaps three, about 9 per cent., are of relatively late Eurasian origin. The rest are probably autochthonous in North America.

COMPARISON OF PLEISTOCENE AND RECENT FAUNAS

Successive faunas in the same region show differences due to three sorts of changes: (1) Immigration of new species; (2) evolutionary advance or differentiation of stocks already present; (3) extinction or migration of groups present in the earlier fauna.

It has been shown that immigration of new species accounts for little if any of the difference between the Melbourne and Recent faunas in Florida. Evolutionary differentiation has doubtless brought about some changes in subspecies but seems to have affected the species but little or not at all. The very great difference between these two successive faunas is thus almost or quite entirely due to extinction or emigration. The Recent fauna is simply a part, probably about two fifths, of the Pleistocene fauna. It has little or nothing that is new, except its poverty and limitation.

The problem of derivation of the Recent fauna thus becomes one of extinction. The recent fauna owes its character wholly to the fact that some Pleistocene species became extinct while others survived. This extinction had a differential character, affecting some groups more

than others, entirely removing three orders, for instance, while the rodents were comparatively little affected. Also within the orders it spared some species and removed others apparently equally fitted for survival. This differential effect of extinction in determining the character of a fauna is worthy of tabulation and study. Its general nature in this case is made clear in the following table.

Order	Pleistocene species not present in the Recent fauna	Percentage of total Pleistocene-Recent species of this order
Marsupialia	0	0
Insectivora	0	0
Rodentia	7	29
Carnivora	9	45
Edentata	7	100
Perissodactyla	6	100
Artiodactyla	10	91
Proboscidea	4	100

Certain of the species belong to genera now extinct everywhere, while others represent more or less local extinction of groups surviving elsewhere. The extent to which this extinction was local may be judged from the following table.

Order	Total known in Pleistocene	Genera extinct	Genera living but not in Florida	Known Pleistocene genera living in Florida
Marsupialia	1	0	0	1
Insectivora	3	0	0	3
Rodentia	16	2	4	10
Carnivora	14	3	2	9
Edentata	6	5	1	0
Perissodactyla	2	0	2	0
Artiodactyla	7	4	2	1
Proboscidea	3	3	0	0

Of the living genera not now present in Florida, seven (*Thomomys, Ondatra, Synaptomys, Erethizon, Vulpes,*

Canis [*Thos*], and *Bison*) have ranged north and west of Florida in recent times, some rather close to the state, others (as *Thomomys*) not within hundreds of miles. Of these, one (*Erethizon*) is of ultimate southern origin but now ranges into the far north and has become adapted to a zone no longer present in Florida. Two (*Ondatra, Synaptomys*) are probably of Eurasian or far northern origin. *Ondatra* lives in the southern part of its range in conditions also found in Florida. *Synaptomys* is now confined to zones (Boreal to Upper Austral) not extending into this state. The other genera of this group are probably autochthonous and still occur in zones present in Florida.

Two genera (*Tatu, Tagassu*) occur almost directly west of Florida, in some cases under conditions present in this state, and also southward into South America. Two (*Hydrochœrus, Tapirus*) occur only in the neotropical region in this hemisphere. Of these southern forms, two (*Tatu, Hydrochœrus*) belong to families of neotropical origin, although they range more widely northward in the Pleistocene, while two (*Tagassu, Tapirus*) are of holarctic origin but survive only in the south.

Among rodents, the Pleistocene fauna included most (perhaps all) of those still living, and also three small rodents (a pocket gopher, muskrat and mouse-lemming) ecologically somewhat similar to those still present in Florida and of genera surviving elsewhere. One, the porcupine, was larger, also survives elsewhere, but has no analogue in the modern fauna. Three (giant beaver, two large capybaras) were very large, larger than any rodents in the world to-day, and have no analogues in the modern fauna of Florida or of North America as a whole.

Among carnivores, in addition to recent types, there were three specific variants (a raccoon, a gray fox and a cougar) of genera common in the recent fauna. There were two (a red fox and a coyote) not now very closely paralleled in the Floridian fauna but of genera still com-

mon elsewhere in North America. There were three (short-faced bear, true giant cat, saber-toothed cat) not very closely paralleled in North America now, and on the whole larger and more powerful than any living Floridian carnivores. There were seven species of edentates, referred to three genera of ground-sloths, one of glyptodonts and two of armadillos, which are without any parallel in the modern fauna except for the smaller armadillo which is not unlike some now living but not in Florida.

There were horses and tapirs, three species of each, wholly absent in the Recent fauna of Florida although of genera surviving in very distant regions. There were peccaries in great variety (probably three genera and six species) and three species of camels, all totally unlike any Recent Floridian mammal. There was a bison, different from the modern bison which ranged not far from Florida but not into that state.

Finally there was a mastodon and three mammoths which are also wholly unlike anything in Florida to-day.

The richness of the Pleistocene fauna of Florida in species was extraordinary. It was probably about equal to, and perhaps even greater than, the average variety in single faunas throughout the Tertiary. It was certainly greater than the usual variety in modern faunas of comparable areas.

In view of the marginal, peninsular nature of Florida, its limited size and its relatively uniform physiographic and climatic conditions, this complexity of fauna is all the more surprising. Yet it seems probable that these very factors, which to-day militate against its faunal variety, contributed to this in the Pleistocene.

The outstanding events which led up to the Pliocene and early Pleistocene culmination of mammalian life were a series of world-wide migrations. Interchange of mammals took place between North America and South America on one hand and North America and Eurasia on the other. The result was a continental fauna very

rich in variety and more heterogeneous in origin than most of the earlier faunas. This enriched animal life, in which Florida shared, had probably reached a natural balance by early Pleistocene time.

The outstanding event of the Pleistocene was, of course, the glaciation. The glacial front stood far north of Florida, and its effect on that state was largely indirect but probably profound. Climatic change doubtless occurred, but was probably not great. Hundreds of miles from the glaciers and surrounded, as now, by tempering waters on three sides, in the path of tropical currents, it is not to be supposed that the temperature here was lowered as much as that of the glaciated areas. The isotherms would not be pushed uniformly southward, but rather would be crowded together, producing a steeper temperature gradient between Florida and Canada.

This is largely borne out by the fauna itself. Only one known Pleistocene mammal is of boreal affinities (*Synaptomys australis*), and this is a distinctive species of a genus which does range well into the Upper Austral Zone to-day. On the other hand, a distinct element of the fauna has decided tropical affinities.

The advance of the ice-sheet made a large part of North America almost uninhabitable and another large part inhospitable to most mammals. Southward movement of the mammalian hordes was inevitable. Even aside from the animals which might migrate to great distances, a surging impulsion would be transmitted so that species or individuals which never lived near the glaciated areas would yet be urged southward by the pressure of concentrated populations north of them.

America east of the Rocky Mountains is bifid. An open channel leads into the great expanse of Mexico and hence eventually into South America. Another (and rather less important) route leads into Florida, but this is a blind alley. One must thus envision Florida as a sort of a trap or pound where the animal population

was significantly increased both in number and in variety by the creatures urged on and loosely confined by biotic pressure from the north and with no escape in any other direction. Here in Florida they found an asylum, climatically more favored than almost any other part of the continent, but a scene of the bitter warfare which always follows overpopulation. The favorable conditions here and the absence of the much more severe stress of the northern region probably led to the survival of many species to a much later time than nearer the glaciated areas. There is considerable evidence for this view, quite aside from the theoretical probability of such an occurrence.

Extinction did finally visit the Floridian fauna with results even more dire than elsewhere on the continent. If everything favored variety of fauna in the Pleistocene, there is evidence that the contrary was true after the Pleistocene. Recent Florida is actually less favorable to variety in species than many more continental parts of the United States. Its thirty-three recent species are less numerous than the average for similar areas in this country. Its Pleistocene fauna includes eight genera which survive elsewhere in the United States but are extinct in Florida. Only one Pleistocene genus (*Neofiber*) survives here and not elsewhere,[1] and as yet there is no positive evidence that it ever occurred elsewhere, although this is highly probable. This poverty of the recent fauna is almost as striking in its way as the richness of the Pleistocene fauna. There seems, for instance, to be no purely environmental reason for the absence of the bison in the recent fauna.

Causes of Extinction and Their Action in Florida

The disappearance of a species or large group may be due to any of three causes: transformation into something else, emigration to a different region or racial

[1] It does transgress the political boundaries, but is confined to extreme southeastern United States.

death. In the first case, the group effectually disappears as such but is not really extinct. In the second, it merely changes its location but may be considered in one sense as locally extinct. In the third, unqualified extinction has taken place.

In the present example, it has been shown that the degree of evolution between Melbourne time and Recent was largely or wholly subspecific. No species are known which disappeared because of transformation into other species.

It might be supposed that the presence of genera in the Floridian Pleistocene which survived elsewhere but not there gave evidence of extensive emigration, but this is not true. It is probable that numerous individuals and even whole species did in effect move northward at the close of the Pleistocene and leave Florida, but if so one is at a loss to cite more than one probable example (*Ondatra zibethica*), and this can hardly have been an important factor in modifying the faunas as known. For the most part it is known that these genera did inhabit large areas in the Pleistocene, including much or all of their present ranges and also Florida and other areas outside their present ranges. Where adequately known, the Floridian species of these genera, except *Ondatra,* have proved to be distinct from the living species. These are not examples of emigration but of restriction of range through the true extinction of marginal species. The red fox may be taken as one example. There was a red fox in the Pleistocene of Florida. None lives in the state now, but a red fox still occurs farther north along the Atlantic Coast. The animal did not, however, migrate northward at the close of the Pleistocene. The eastern red fox, *Vulpes fulva,* was already present within its recent range, and the Florida species, *V. palmaria,* became extinct.

The disappearance of so many species from Florida was thus due to their true extinction. The causes of ex-

tinction, so much discussed, are manifold. They may be classified roughly as follows:

I. Inherent causes.
  a. Independent of environmental change.
    1. Disadvantageous specialization.
    2. Loss of racial vitality, lowering of reproductive power.

  b. Related to environmental change.
    1. Highly specific adaptation and loss of adaptability.
    2. Lowering of reproductive power through external influence.
    3. Special susceptibility to various external influences through size, physiology, habits, etc.

II. Physical environment.
  a. Direct.
    1. Directly unfavorable climatic and physiographic changes.

  b. Indirect.
    1. Restriction of range.
    2. Physical influence on physiology.
    3. Physical influence on biotic environment.

III. Biotic environment.
  a. Loss or restriction of food supply.
  b. Introduction or multiplication of enemies (including parasites, poisons, etc.).
  c. Intensification of competition or introduction of new and able competitors.

The list is not complete or detailed, but it includes most of the supposed factors of extinction. All are interrelated, and extinction through the operation of a single and simple cause rarely has occurred. The so-called inherent causes are particular: they influence the extinction of certain species rather than others. The environmental causes are general: they influence a fauna as a whole and cause those susceptible for inherent reasons to become extinct.

It has been argued (most recently by Tolmachoff) that the principal if not the only cause of extinction is inherent and independent of external factors. A species or a race is supposed to grow old, as do individuals, to expend its energy, to lose adequate powers of propagation. Two related but really distinct causes are cited:

the development of races with characters which, regardless of uniformity of environment, are actually disadvantageous—the giant Irish deer often given as an example—and the depletion of racial vitality to the impairment of adequate fertility. It is obvious that non-advantageous variations or mutations do occur, but it is an axiom of natural selection that if they are actually disadvantageous and without compensating correlation with other characters they will not be perpetuated to the point of becoming fixed features of a race. The reality of evolutionary momentum or orthogenetic evolution to the point of dangerously disadvantageous specialization with uniformity of other conditions has been challenged and is certainly not proved.

Whatever the case in other faunas, it hardly seems possible to apply this doubtful cause of extinction to the apparently advantageously specialized mammals of Florida. The nature of these extinct mammals also seems to me to be opposed to the theory that senility in itself was a primary factor in this case, whatever may have been true in other instances. The main argument seems to be that animals that were highly specialized and racially old have become extinct, and that external factors, in the opinion of the authors advancing the argument, were inadequate. The analogy with individual life is very precarious if not absolutely false, and the alternative explanation that extinction was due to inadaptability under changing external conditions seems adequate and is applicable to every case of this sort.

Certainly in the specific case of Florida there is much evidence against senility being the main or only cause of extinction and no good evidence for its being a real factor at all. Was *Procyon nanus* depleted of inherent vital force while its larger and equally specialized very close relative and faunal associate *Procyon lotor* was well able to flourish indefinitely? Why were species of *Equus* in North America racially older and less prolific than species apparently almost identical in size and

structure in the Old World? Examples of the insecurity of this assumption could be greatly multiplied from this fauna alone. Two types of animals, the extinct very large-horned bison and the mammoths, might be adduced as evidence for this view. They are not, like some of the other examples, definitely opposed to it, but it seems to me that there is no positive evidence whatever that *Bison latifrons* or *Parelephas columbi,* which became extinct, were in an infertile racial old age while *Bison bison* and *Elephas indicus,* which survived, were racially more productive and vigorous.

The almost simultaneous extinction of two thirds of the Pleistocene fauna can hardly be considered as an inevitable event regardless of external circumstances. The selection of certain species rather than others for extinction must reasonably have been due in most cases or in all to differences between the species, hence to their own characters, but the influence which made these different characters fatal in so many cases must have been external. Aside from other considerations, the mathematical probabilities are enormously against so many and such extremely various animals reaching their period of existence at a time so definite and limited from a geologic point of view, and these probabilities greatly favor the action of some external factors more strongly at this time than at any other.

The problem is dual. What were the external factors that impelled faunal impoverishment? And what were the individual characters that directed extinction to certain species?

The external factors present have already been suggested in part. So far as the evidence is available they were as follows:

I. Physical.
  a. Geographic and physiographic.
    1. Limited area. Except probably at its extreme southern end Florida was not smaller in Melbourne or post-Melbourne time than now, but this was a small area for the abundance of mammals then present as a result of other causes.

2. Imperfect drainage. There has probably been some change in this respect, as there is evidence of slight elevation and new incised drainage lines since Melbourne time.

b. Climatic.

1. Climatic change. In Florida itself the change since Melbourne time has probably been very slight. The average temperature may have been slightly lower, although this is not clear, but it probably has not been significantly higher. There is little evidence bearing on changes in precipitation or humidity.

c. Biotic.

1. Intensification of struggle through enlarged population.

2. Partial duplication of adaptive types through mingling of species of different geographic origin.

3. Restriction of food supply. There is no evidence of the restriction of plant food beyond that probably present in Melbourne time, and now present, which is nearly a maximum. The extinction of numerous herbivores would constitute a very decided restriction of carnivorous food supply.

4. Introduction of new enemies. The introduction of new parasites or pathogenetic organisms or of poisonous plants is a possibility on which there is no evidence. Within the fauna itself there are no enemies, with one exception, that had not been present for a considerable time and hence were not probably in faunal equilibrium. The important exception is man, most powerful and destructive of all vertebrates. There is now good evidence that he was not present much before Melbourne time, but did appear just before the most marked faunal impoverishment.

5. Indirect influence of faunal disturbance elsewhere on the continent. There is evidence that many of the groups surviving in Florida in Melbourne time had previously become locally extinct over large areas. This would have a marked local influence in the diminution of herds, in lessened opportunities for cross-breeding, in the liability to total extinction through purely local or temporary causes, in the intensification of struggle with enemies and possibly in other ways.

These circumstances, acting together, indicate the reasons for extinction of so many animals at this particular time. All animals were undergoing stress which resulted in the elimination of those most definitely affected.

The intricately correlated and in part unknown factors which led to the extinction of some species rather than others can not be fully elucidated, but are suggested to some degree by their characters.

The only case in which climatic change can be considered as a definitely possible factor is that of *Synaptomys*. Even in this case climate was probably secondary to other causes. In Florida this animal was in competition with numerous small cricetines and microtines and preyed upon by numerous carnivorous vertebrates. Its survival to the northward may be due to its adaptation to particular and specialized conditions, giving it sufficient advantage in competition and sufficient protection from enemies to permit survival. They are obscure and, usually, relatively rare animals, living principally in a cold moist habitat, in the southern part of their range almost wholly in cold sphagnum bogs. Similar habitats may or may not have occurred in Florida in Melbourne time, but do not occur there now.

Certain of the Pleistocene species of Florida represented essential duplication of adaptation. The clearest examples are the association there of *Thomomys* and *Geomys* and of *Ondatra* and *Neofiber*. These genera all survived to the recent, but *Thomomys* is not now found with *Geomys,* nor do *Ondatra* and *Neofiber* occur in the same area now. Just what subtle local advantage decreed that in Florida *Geomys* and *Neofiber* should be successful is not clear. Equally striking and similar in significance are apparent cases of the existence of two closely related species of one genus where but one is now present: *Urocyon* with the Pleistocene species *U. cinereoargenteus* and *U. seminolensis,* of which only the former survives, and *Procyon* with *P. lotor* and *P. nanus,* only *P. lotor* surviving. The cougars are not yet well known, but it is possible that they too included two closely related species, and only one, *Felis coryi,* has survived.

Then there is a duplication less complete and probably to be considered rather as a contributory than as a pri-

mary cause of extinction. *Arctodus floridanus* belongs here. Its mode of life must have resembled that of *Euarctos floridanus* somewhat, but not to the point of truly lethal competition. The same would be true of *Ænocyon ayersi* with respect to *Canis floridanus,* if indeed the latter wolf was present in the Pleistocene. *Vulpes palmaria* and *Canis* (*Thos*) *riviveronis* doubtless met competition to some degree from the gray fox, wolf and other carnivores, although they are well able to survive such competition elsewhere.

The examples just given suggest a crowding into Florida of species which would not ordinarily develop in such a limited area and probably would not indefinitely remain distinct there together. These abnormal conditions were probably due to the peninsular nature and the position of Florida and to the impulsion of unfavorable climatic and other conditions in the north.

The decisive influence in the extinction of the larger predaceous carnivores, *Canis* (*Ænocyon*) *ayersi, Felis veronis* and *Smilodon floridanus,* was probably secondary: the failure of adequate food supply for which they were specifically adapted, through the extinction of most of the larger herbivores. Temporary diminution of plant food during a season or two may have further jeopardized herbivores already in a precarious position due to other causes, but this is purely speculative. There is no evidence of any general or long-continued diminution of their food supply.

There remains a large part of the fauna probably not much affected by these more obvious partial causes. Among rodents, the giant beaver and capybaras belong here; among edentates, all the known forms, ground-sloths, armadillos and glyptodonts, and among ungulates, the horses, tapirs, peccaries, camels, bison, mastodon and mammoths. Their extinction is particularly striking and puzzling and was probably due to causes still more complex than those affecting the other mammals. Unlike the species so far discussed, they apparently could live

in Florida under Recent conditions, and yet there are no mammals there which parallel them at all closely in adaptation.

These diverse animals do possess some important features in common. All are herbivorous. All are large, the white-tailed deer being the only living herbivore as large as any of them, and the smallest, *Tatu bellus,* a large armadillo twice the size of the species living in the Southwest. With the probable exception of the edentates, all are communal or even highly gregarious. Most of them apparently thrived best in a rather warm climate and some were definitely subtropical. The rodents are water-loving forms, but the others are relatively intolerant of swampy or very wet situations. All are unusually attractive prey to carnivores, including man, and many of them are attractive to primitive man for special reasons, such as desirability of their hides, pelts or teeth. Most of these characters can be shown to have had a possible influence in the extinction of these groups.

Large size exposes its possessors to especial danger in times of stress. Large animals multiply more slowly, have a longer juvenile period, require more food and are relatively fewer in number in a given area.

Communal or herd life is somewhat analogous to large size in that it is especially advantageous under normal conditions but may become peculiarly disadvantageous under abnormal conditions. In some cases the safety of the individual, especially when young, is dependent on the functioning of the herd. In such cases a temporary decrease in herd size may initiate extinction while an equal temporary diminution in the number of individuals of the solitary species would have no permanent effect. Communal or gregarious animals are much more susceptible to epidemics. Restriction of range or separation of groups is more likely to lead to degeneration. With the vast inland region populated by similar and accessible groups, the vitality of the species in Florida would

be maintained by intermigration and marginal contact. With the removal of this background, or with loss of contact with it, as seems definitely to have occurred at the end of the Pleistocene, the distinctly limited Floridian herds were thrown on their own resources with degeneration as an almost inevitable result. Finally, animals that live in groups are especially subject to the depredations of man as seen in the methods of mass slaughter known even to early man and also to the disruption of the necessary communal life by the killing of leaders or males.

Various factors may be considered as possibilities or contributing causes of perhaps minor import. The introduction of new parasites or diseases or poisons is distinctly possible, although hardly a complete or general explanation in view of the diversity of the animals affected. Poor drainage and unusually wet seasons may have had an indirect influence in the still greater restrictions imposed on available range and possibilities of free migration. Change in average temperature was probably not great, but in these particular animals even a short succession of unusually severe winters might be very dangerous to the race.

The groups which did so dramatically and, it would seem, so mysteriously become extinct were on the whole just those in which, under the conditions at this time and in this area, the combined action of various adverse circumstances might lead to a crucial diminution in numbers. In animals of different character (as those that did survive in Florida) or in similar animals under different conditions or not subjected to this combination of stresses (as close relatives that survived in other regions) this diminution might be purely temporary. These particular groups were unduly susceptible to permanent effects from temporary causes.

SHORTER ARTICLES AND DISCUSSION

SPERMATOPHORES OF AN OREGON CRAYFISH

Of the seventy or more sorts of crayfish found in North America almost all are of the genus *Cambarus* and only a few are of the genus *Potamobius* (commonly known as *Astacus*) and these are restricted to the western part, while *Cambarus* occupies the area of the Mississippi and easterly as well as Mexico and lower Canada.

While the genus *Cambarus* is peculiar to this region, the genus *Potamobius* has several species in Europe and in western Asia where no other genera are found. In the east of Asia are some crayfish referred to a genus *Cambaroides* that has some resemblances to each of the other two genera, *Potamobius* and *Cambarus*. All the crayfish known south of the equator are of many genera differing from these north of the equator.

Underlying any adequate explanation of the peculiarities of this geographical distribution should be a better understanding of the differences between these several genera and species.

As far as known, *Potamobius* differs from *Cambarus* internally in valvular arrangement of the heart and in slight numerical diversities in gills. Externally, however, there are marked differences in the secondary reproductive organs, because in the male *Potamobius* has more simple and vaguely elaborated organs for transferring the sperm as compared with the highly specialized and perfected organs of *Cambarus* that differ markedly in different species. Moreover, the female *Potamobius* is generalized as having no organ into which the sperm may be received, while the female of *Cambarus* has in each species a differently fashioned specialized external pocket in the shell into which the sperm is placed by elaborate, strenuous and precisely adjusted exertions of the male.

It is now well known that the "annulus ventralis," formerly known only as a structure relied on as a specific character in *Cambarus*, is the sperm receptacle peculiar to *Cambarus* and not found elsewhere, though an analogous structure occurs in the common American lobster.

Huxley and others described the sperm transfer in the genus *Potamobius* of Europe in which there is no receptacle and the sperm is deposited in capsules or spermatophores on the parts of the shell of the female over which the eggs will travel upon being

laid, when the sperms are supposed to emerge and to become attached to the eggs.

As was to be expected, we find this same diffuse application of spermatophores to take place in some American crayfish of this genus *Potamobius*. In 1927, some forty living and forty dead *Potamobius trowbridgei* received from Professor Lawrence E. Griffin, of Reed College, Oregon, were apparently in breeding condition at that season, November, since two dead females had still some few eggs attached to the pleopods, and one dead male spermatophores attached to the ends of the first stylets.

After a few days in captivity one of the females was found dead with conspicuous white spermatophores attached to the ventral surface. The spermatophores were slender white threads of variable lengths, but an average might be about five millimeters long and about $\frac{1}{4}$ mm thick. They were attached chiefly in two dense masses, but with scattering exceptions as follows. On the bases of the large antennae there were three on the right and five on the left, eight on the head in all. Just anterior to the oviduct openings on the thoracic surface depressed below the legs there were two small spermatophores on the right and one on the left; these eight plus three anterior to the oviducts would be of no use as they would not have access to the eggs which pass posteriorly from the oviducts to the abdominal pleopods. Just posterior to the oviduct openings in the depressed thoracic surface behind the leg base, there was one spermatophore on the right and one on the left and on the median sternum between the fourth legs there were four.

Coming now to the main masses: On the sternum between the fourth and fifth legs there was a large mass made by adhesion of thirty-three spermatophores, twenty in the main central mass with five on the right between the leg bases and eight on the left between the leg bases. All these presented distinct tips that were counted while the attached bases were stuck together more or less indistinguishably.

On the movable sternum of the thorax just anterior to the first abdominal pleopods there was a second large mass of spermatophores, forty-one in all, thirteen on the right, twenty-five on the left and thirteen on the middle. Thus the entire number of spermatophores counted was ninety-one, and of these all but eleven were placed posterior to the oviduct openings and hence could have been of use if their sperms issued properly to connect with the eggs.

How long these spermatophores retain the sperm and by what means they are emptied for the fertilization of the eggs is not directly known. The suggestion was made by Leuckart that by the gradual change of the wall of the spermatophore, (a secretion from the lining cells of the deferent duct), a discharge of sperms might be caused; but this would need be accurately timed. Very likely, as surmised by Meyer, it is the action of some secretion of the female at the time of laying eggs which induces the emptying of the spermatophores. In any case there may well be considerable loss of sperm. From this point of view it is of interest to determine approximately the number of sperms available in all these spermatophores.

Previously it had been computed for *Cambarus limosus* that the numbers of sperms deposited in the annulus ventralis or sperm receptacle was in the neighborhood of 60,000 to 100,000, and that after laying some 10,000 of these might still remain unused in the inmost recess of the receptacle. As the female lays but 200 to 600 eggs at once, according to age and size, there is here ample provision for each egg to become fertilized and in fact it was seldom, in captivity, that all the eggs were not fertilized. In the male the length of the deferent duct was 60 mm, and it might contain on both sides 2,000,000 sperms, sufficient to fertilize the eggs of many females.

We found the deferent duct in *Potamobius trowbridgei* longer, as might be expected in this larger animal, but disproportionally longer, some 230 mm, and thus the male may well supply more sperms than is the case in the above *Cambarus*.

The number of sperms actually found upon the above female *Potamobius* was estimated to be one half million as follows. In an average spermatophore teased into bits in drops of water and then dried on glass, stained with Haidenhain's haematoxylin, the sperms were counted as 5,412. In water the sperms previously densely compacted in spheroidal form expand the ray-like appendages and glide out along the glass and thus they may be counted. The rays were very long, about twenty, but apparently variable in number and thus much more numerous than yet observed in *Cambarus*.

Assuming that these findings recur again in other species of *Potamobius* and of *Cambarus*, we may tentatively add to the other generic differences differences in lengths of deferent ducts, differences in numbers of sperm arms, and differences in numbers of sperms supplied at one time by the male.

With increasing knowledge of the generic differences it becomes increasingly difficult to picture just how such differences may have arisen; as modifications of some common basis; or derivation of one genus from the other, since geographical distribution leaves little doubt that *Cambarus* is the newest genus of crayfish.

The existence of a sperm receptacle in *Cambarus* is apparently correlated with an economy of sperms, since the numbers used for one egg-laying are much smaller than in *Potamobius,* where the spermatophores are scattered and not concentrated within a small receptacle; moreover, the central position of the receptacle seems better to assure the contact of the sperm with the issuing eggs than the wide spreading of the spermatophores on the outside of the shell, since the eggs tend to glide along the middle line, though emerging right and left.

It is then possible to regard the invention of the sperm receptacle as a gain in economy and a useful improvement that might, conceivably, be of some moment to the race; hence natural selection might have had a hand in its preservation.

In brief, the accuracy of aim in sperm transfer may have enabled *Cambarus* to avoid some of the loss of materials caused by the scattering methods of *Potamobius.* Other characters of these two genera, such as the slight differences in numbers of gills and the numbers of arms of the sperms, can scarcely be thought of as in themselves of any survival value, and raise the question whether we shall regard this whole complex of secondary sexual organs and their functions as arising from unknown variations in supposed genes producing results in part seemingly significantly useful.

Pending experimentation we remain quite ignorant of any explanation of the causes for such generic differences as those separating *Potamobius* and *Cambarus.*

E. A. ANDREWS

JOHNS HOPKINS UNIVERSITY

CHROMONEMATA IN SOMATIC AND MEIOTIC MITOSES

STUDIES of root-tip mitoses of *Allium cepa* and of *Iris versicolor* reveal chromosome structure and chromonemata behavior essentially similar to that previously described for somatic mitoses of *Tradescantia pilosa* (Kaufmann, 1926*a*) and *Podophyllum peltatum* (Kaufmann, 1926*b*). Sharp also has reported the presence of chromonemata in the various phases of root-tip mitoses of *Allium cepa,* as part of a more extensive investigation

of large somatic chromosomes (Sharp, 1929). In my studies of *Tradescantia* and *Podophyllum*, the anaphase chromosomes were found to be composed of two visibly dissimilar substances, designated as chromatic and achromatic. The chromatic material exists in the form of a pair of spiral threads, embedded in the achromatic matrix. The threads or chromonemata persist through the telophases and the interphases, although discernible with difficulty in the latter stages, on account of the building of interchromosomal anastomoses. In the succeeding prophases each of the parallel threads was observed to give rise to two new chromonemata, which are destined to separate at the second succeeding anaphase. Metaphase chromosomes of somatic mitoses are therefore tetrad, with respect to the chromatic material, since each half chromosome contains a pair of chromonemata.

The race of *Iris versicolor* used has a diploid chromosome number of about sixty-eight. This proved good material for the study of chromonemata in smaller chromosomes. Chromonemata were sought also in the small chromosomes of the root-tips of the ferns *Polypodium incanum* and *Adiantum pedatum*. In these it is possible to identify the spirals in protruding ends at anaphases, and occasionally to recognize duality during these stages and the telophases. Late prophases give some indication of the spirals within each of the half chromosomes. Up to the present it has been impossible, however, to determine either the time of origin of the chromonemata, or their behavior during the phases of mitosis not mentioned. Fragmentary evidence of detached phases is suggestive, nevertheless, of the organization more readily discernible in larger chromosomes throughout the mitosis.

The behavior of the chromonemata in meiosis is the particular concern of the present preliminary report. The following account is based primarily on observations of microsporogenesis in *Tradescantia pilosa* and *Rhoeo discolor*, although the chromonemata have been traced through meiosis in the additional species, *Podophyllum peltatum*, *Iris versicolor*, *Pinus echinata*, *P. palustris* and *P. taeda*, and have been seen at metaphase of the first division in *Allium neopolitanum*, *Gasteria* sp., and *Tradescantia virginiana*. The occurrence of a "bouquet stage" in *Tradescantia* and *Rhoeo* has been reported (Kaufmann, 1925), and the tetrads of the first maturation division of these two species have been figured.

At the anaphases and the telophases of the last pre-meiotic division the chromosomes exhibit the parallel chromonemata, as reported in an earlier paper (Kaufmann, 1926*a*). The chromonemata persist through the subsequent interphases, although the space between them often is obscured. That duality exists is indicated by the split along the turns of the coil occasionally seen in leptotene-threads. In *Tradescantia,* synchronous with the extension of the leptotene-threads, the plasmosomes flatten against the inner face of the karyotheca. There they form an "attachment plate" for the elongating threads, which accordingly are polarized, and loop out into the nuclear cavity initiating the "bouquet stage." Synizesis, seen in some preparations of this stage, can be controlled by proper fixation.

At regions of maximum extension the leptotene-threads are smooth, except for such chromomere-like swellings as are due to the tightening of the coils incident to the elongation of the threads. The appearance of a spireme composed of discrete particles or chromomeres suspended on a lighter staining thread was encountered, but better preparations of these stages showed only nodal points caused by sharp turns of the coils. In many preparations it is apparent that complete elongation of the homologous threads is not always prerequisite to their pairing. Nuclei in the amphitene-condition evidence this most strikingly, the coils appearing with diagrammatic clarity both in the paired and the unpaired portions of the chromosomes. This observation lends the strongest support to the parasynaptic interpretation, for it is possible sometimes to determine that pairing is side-by-side between chromosomes which are longitudinally split (a split referable to the prophase of the last pre-meiotic mitosis.) Verification of parasynapsis is of especial interest in *Tradescantia* and *Rhoeo* because of the ring or chain formation, most strikingly seen at metaphase, and involving some or all of the bivalents. The observations here reported support the conclusions of other workers that parasynapsis is tenable with subsequent end-to-end linkage.

During the ensuing prophase stages the chromonemata become increasingly conspicuous, concurrent with chromosome shortening and condensation. At metaphase each homologue of the bivalent contains two spirally arranged, parallel, chromatic threads embedded in an achromatic matrix.

As the anaphase chromosomes move toward the poles (or slightly earlier) the chromonemata separate, the achromatic

matrix splitting simultaneously. The majority of preparations of these stages reveal a single spiral thread within each member of the dyad, but occasional smears show duality. That the latter is probably the true structural condition is indicated at the time of telophasic transformation, when dissolution of the achromatic matrix exposes more fully the two chromonematic skeletons which are destined to separate at the first post-meiotic anaphase.

During interkinesis the chromonemata may be obscured temporarily by anastomosing. Prophase and metaphase chromosomes of the second division, even at the time of maximum extension, show pronounced internal spirals. Such chromonemata can be traced through the succeeding anaphases and telophases.

Diagrams illustrating the above interpretation of somatic and meiotic mitosis are inserted as Figs. 1 and 2.

BERWIND P. KAUFMANN

DEPARTMENT OF BOTANY,
UNIVERSITY OF ALABAMA

LITERATURE CITED

Kaufmann, B. P.
1925. AMERICAN NATURALIST, 59: 190.
1926*a*. *American Journal of Botany*, 13: 59–80.
1926*b*. *American Journal of Botany*, 13: 355–363.
Sharp, L. W.
1929. *Botanical Gazette*, 88: 348–382.

A MODIFIER OF PIEBALD SPOTTING IN MICE[1]

THE F_2 offspring of a cross between a strain of black-eyed-white mice and a self-colored strain exhibited a great variety of spotting patterns. Practically all the spotted F_2 animals were tested for the black-eyed white gene by mating them to animals known to contain that gene. The production of anemic offspring (WW animals, *cf.* De Aberle,[2] 1925) by the tested animal immediately identified it as a carrier of the black-eyed white gene. By this process there were identified also a number of spotted animals that did *not* carry the gene but were ww in constitution. A few of these proved to be wwSs in constitution because when mated together they produced a number of self-colored animals. Furthermore the spotting of such animals was very slight indeed, being confined to a few white hairs on the forehead or belly and slight white spotting on the toes and tip of the tail. The wwss

[1] From the Bussey Institution, Harvard University, Forest Hills, Boston, Massachusetts.

[2] S. B. De Aberle, AMER. NAT., 59, 327–335. 1925.

animals proved to be easily separable into two classes. One called dark-faced was characterized by practically no face spotting, definite white spotting on toes and tail and a varying amount of belly spotting. In this class the maximum amount of face spotting consisted of a small forehead blaze which might occasionally extend in a narrow white line to the nose. The "belt" of white spotting observed in certain strains of piebald mice was not definitely present in these animals though in certain animals a slight amount of dorsal white was observed in the position of the "belt." The second class resembled the first in all details except that it contained definitely white-faced animals. The white face was the type commonly observed consisting of a V-shaped area of white hairs with the point of the V located between the ears. In a number of the animals, small, irregularly distributed areas of dark-colored hairs were located on the white face. In a few cases the distinction was not completely clear between a somewhat extended forehead blaze and a white face with a large number of dark areas. It was decided, therefore, to undertake a certain amount of inbreeding in an attempt to obtain clearly contrasting types.

As soon as the inbreeding was begun it became evident that white-faced animals bred true with remarkable consistency. After about four generations of brother-sister matings of white faced by white faced, 317 animals were produced in 76 litters and 314 of these were definitely white-faced. The three exceptional individuals were derived from parents which possessed much reduced face spotting that might have represented rather large forehead blazing, and none of the three was completely dark-faced.

The matings of dark-faced animals *inter se* fell into two classes: (1) those which produced both dark-faced and white-faced offspring; (2) those which produced only dark-faced offspring. After about four generations of brother-sister mating 58 litters of class (2) were produced containing 272 dark-faced young. Concurrently there were produced twenty-six litters of class (1), 127 animals of which were dark-faced and 49 of which were white-faced. On the expectation of a 3 to 1 segregation of dark-faced from white-faced we should have 132 dark-faced to 44 white-faced; the deviation is 5, the probable error, 3.87.

Furthermore, certain of the matings consistently involved the breeding of white-faced brothers to dark-faced sisters and *vice versa*. In 47 litters from such matings 253 young were pro-

duced, of which 129 were dark-faced and 124 light-faced. The deviation from a 1 to 1 ratio is 2.5, the probable error, 4.65.

It was, therefore, concluded that white-faced spotting was due to a single recessive modifier independent of the gene for piebald spotting (ss) but exerting its effect only in the presence of the piebald gene. This recessive modifying gene may be called *l*. White-faced piebalds are therefore *ll ss*, and dark-faced piebalds either *Ll ss* or *LL ss*.

It might be objected that the 1 to 1 ratio obtained for matings of white-faced piebalds to dark-faced piebalds is misleading, inasmuch as certain of the dark-faced animals might have been LL ss in constitution, and therefore an excess of dark-faced animals should be expected. It must be remembered, however, that the data for these crosses were derived from *consistent* brother-sister matings of white-faced animals to dark-faced sibs, so that one might expect an excess of dark-faced young only in the first brother-sister generation. Most of these data are from the 2nd, 3d and 4th brother-sister generations. Similarly, certain of the dark-faced animals in the group of dark-faced matings producing only dark-faced young must have been *Ll* in constitution. Certain of these animals did, in fact, prove to be so when they were tested in subsequent generations, producing approximately equal numbers of white-faced and dark-faced young when mated to white-faced animals. By far the larger number, as was to be expected, proved to be homozygous LL when crossed to white-faced animals since all the offspring (103 animals) were dark-faced.

It was decided, after four generations of brother-sister matings, to undertake an independent test of the existence of the gene *l*. Accordingly two types of matings were made: (1) animals from lines of true breeding white-face were mated to self-colored dilute brown mice of the long inbred Little strain; (2) animals of true breeding dark-faced lines were also mated to self-colored dilute brown. F_2, F_3 and backcross progeny were obtained. The results are given in Table I. The dark-faced animals used showed a large amount of belly-spotting. They were easily differentiated from the few F_1 animals that showed a slight amount of white spotting. Most of the F_1 animals were completely self-colored; a few exhibited slight forehead blazes and occasional white spotting on the toes and tail. Such animals appearing in F_2 were classified as self-colored. There may have been some overlapping between these and very dark spotted

dark-faced animals, but this is doubtful inasmuch as practically all dark-faced F_2 animals showed large belly spots often accompanied by dorsal spotting in the position of the "belt."

It is evident from these data that white-faced mice are homozygous for a gene (*ll*) which, in the presence of the normal spotting gene (*ss*), extends white spotting to the face.

Final proof of the existence of this gene was had by inbreeding white-faced F_2 and backcross progeny of these matings. Such animals were bred brother to sister for ten generations. They *always* produced white-faced young. Furthermore, subsequent matings of dark-faced piebalds *inter se* were of two types, those producing only dark-faced piebalds and those producing approximately three dark-faced piebalds to one white-faced.

TABLE I

Generation	Self-colored		Dark-faced		White-faced		Deviation	Probable error
	obs.	exp.	obs.	exp.	obs.	exp.		
F_2 from (F_1 dbr × white-face)	321	325.5	78	81.37	35	27.13	(3:1)4.50 (15:1)7.87	6.08 3.40
F_2 from (F_1 dbr × dark-face)	95	92.25	28	30.75	0	0	2.75	3.24
Backcross, inbred white - face × F_1 (dbr × white-face)	53	50.50	26	25.25	22	25.25	2.50	2.94
Backcross, F_2 white-face × F_1 (dbr × white-face)	18	22	11	11	15	11	4.00	1.94
Backcross, inbred dark - face × F_1 (dbr × dark-face)	53	47	41	47	0	0	6.00	3.25
*F_3 from (F_2 dark-face mated *inter se* [from F_1 dbr × white-face])	0	0	71	73.50	27	24.50	2.50	3.34

* From matings producing at least one white-face animal.

Efforts were made to obtain by inbreeding extremely uniform strains of dark-faced and white-faced piebalds. After four years of inbreeding most of the strains were lost. It is hoped, however, that sufficient satisfactory material remains to permit a further examination of certain variations associated with piebald spotting.

GREGORY PINCUS

THE BUSSEY INSTITUTION,
HARVARD UNIVERSITY

THE CHROMOSOMES OF THE SOUTH AMERICAN OPOSSUM, *DIDELPHIS PARAGUAYENSIS*

Up to the present time no cytological data have been available concerning the chromosome complex of the South American opossum, *D. paraguayensis,* notwithstanding the theoretical interest which would be aroused should the chromosome number and morphology prove to be characteristic of the genus, instead of

being limited to the North American species, *D. virginianus,* investigated by (1) Jordan, (2) Painter, and (3) Hoy and George, and also should it be established that the sex chromosomes correspond to the X-Y type.

In the testis of the young opossum there is an abundance of gonial cells which show the chromosomes clearly. One finds without great difficulty eleven pairs of chromosomes oriented radially in the equatorial plate, among which the different sizes are clearly disinguished. The characteristic shape of the elements is that of a telomitic rod. There are eight small pairs, the

smallest being constituted of two unequal elements, the sex chromosomes. The remaining three large pairs of elements are of almost equal length, although one of them is frequently disproportionately large. As observed by other authors in different genera of marsupials, the sex pair is usually located in the center of the spindle.

Hoy and George found that in some equatorial plates there were sometimes, in addition to the chromosomes X and Y, one or two elements occupying the center of the plate in the same position, but, at least in the cells observed, we have not been able to encounter this arrangement which may possibly be due to a technical artifact.

We agree perfectly with the seriation encountered by Hoy and George in the somatic complexes of different tissues of *Didelphis virginiana*. In the same way *D. paraguayensis* has also three large pairs, seven medians, and the sex pair X-Y.

FRANCISCO A. SAEZ

MUSEO DE LA PLATA,
LA PLATA, ARGENTINA

A METHOD FOR CLEARING LEAVES

THE following method has proved quite effective for clearing and decolorizing whole leaves darkened by preservatives and containing considerable quantities of tannin.

The leaves to be cleared are placed in a test tube and covered with a saturated solution of chloral hydrate for 48 hours or longer. The chloral hydrate is then poured from the material and enough potassium chlorate added to fill the bottom of the test tube to a depth of about $\frac{1}{2}$ inch. The material is then covered with concentrated nitric acid and let stand until the leaves begin to change color. From 10 to 30 minutes is usually sufficient. The nitric acid is then poured out, leaving the potassium chlorate and leaves in the test tube, and the material again covered with a saturated solution of chloral hydrate. In this solution there will be a continuous liberation of chlorine gas and within less than a week the leaves should be quite transparent. If necessary the nitric acid treatment may be repeated.

JAMES C. BATES

DEPARTMENT OF BOTANY,
UNIVERSITY OF KANSAS